510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k060431

B. Purpose for Submission:
New Devices

C. Measurand:
Anti-Ribonucleic acid polymerase III (Anti-RNAP III)

D. Type of Test:
Semi-quantitative, enzyme-linked immunosorbent assay (ELISA)

E. Applicant:
MBL International Corporation

F. Proprietary and Established Names:
Anti-RNA Polymerase III ELISA Kit

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5100, Antinuclear antibody immunological test system
2. Classification:
   II
3. Product code:
   NYO, Autoantibodies, Anti-Ribonucleic Acid Polymerase (RNAP) III Antibody
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The anti-RNA Polymerase III ELISA Kit is a semi-quantitative, enzyme-linked immunosorbent assay (ELISA) for the detection of anti-RNA Polymerase III antibodies in human serum. The test result is used as an aid in the diagnosis of Systemic Sclerosis (SSc) in conjunction with clinical and other laboratory findings. The anti-RNA Polymerase III ELISA Kit is intended for in-vitro diagnostic use.
2. Indication(s) for use:

   Same as intended use.
3. Special conditions for use statement(s):
   For prescription use only.
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450/620 nm.
   Microplate washer

I. Device Description:
Each device contains the following: microwell strips (12x8) coated with recombinant purified RNA polymerase III, two level calibrators (0 and 100 U/mL), goat anti-human IgG, IgA, IgM horse-radish peroxidase conjugate, TMB substrate, wash concentrate, diluent and 1N sulfuric acid stop solution. Positive and negative controls are not supplied with device.

J. Substantial Equivalence Information:

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1. Predicate device name(s):
   Immuno Concepts HEP-2000 Fluorescent ANA-RO Test System
2. Predicate 510(k) number:
   k972145
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Assay format	Semi-quantitative	Same

Differences		
Item	New Device	Predicate
Intended Use	To aid in the diagnosis of Systemic Sclerosis (SSc)	To aid in the diagnosis of multiple autoimmune diseases, systemic rheumatic disease
Technology	ELISA	Indirect fluorescence microscopy
Antigen	Recombinant purified RNAP III	HEP 2000® cells with mitotic figures, stably transfected with SSA/Ro autoantigen
Serum dilution and volume	1:101; 100 µL	1:40; 25 µL
Calibrators	Two levels: 0 U/mL and 100 U/mL	Not applicable
Controls	No Positive and Negative Controls	Six Positive Controls (SSA/Ro, Homogenous, Speckled, Nucleolar, Centromere, Titratable) and One Negative Control
Conjugate	Horse radish peroxidase conjugated goat anti-human IgG, IgA, and IgM with HEPES, Proclin 150 and BSA	FITC Goat anti-human IgG with 0.1% sodium azide
Diluent	PBS diluent with Tween 20 ready to use	PBS buffer powder to make 1 L
Wash concentrate/Buffer	PBS with Tween 20: 10X concentrate	PBS buffer powder to make 1 L
Substrate	TMB	Not applicable
Stop solution	1N Sulfuric acid	Not applicable
Incubation times	60-60-30	30-30
Wash step	4X	PBS Rinse 1X for 10 min. PBS Wash 1X
Result reading	ELISA: O.D. At 450/620 nm	IFA fluorescence microscope at 200X or greater magnification

Differences		
Item	New Device	Predicate
Results interpretation	Negative: <28 U/mL Positive: ≥28 U/mL	Negative: <1:40 dilution Positive: ≥1:40 dilution reported with specific nuclear pattern observed

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
The anti-RNA Polymerase III ELISA Kit measures anti-RNAP III antibodies present in the serum by ELISA. Diluted Calibrators, controls, and patient serum are added to microwell coated with recombinant purified RNAP III antigen, allowing anti-RNAP III antibodies to react with the immobilized antigen. After washing to remove any unbound serum proteins, horseradish peroxidase conjugated anti human IgG, IgA, IgM is added and incubated. Following another washing step, the peroxidase substrate is added and incubated for an additional period of time. Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The assay can be semi-quantified by measuring the reaction photometrically and plotting the results.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility*:
   The intra-assay reproducibility was determined by testing 6 samples 8 times on 3 assays with 3 lots. Two samples with high anti-RNAP III concentrations (61.1 and 150.6 U/mL) had %CVs of 2.0 and 3.4 %, four samples close to the assay cut-off (14.6-32.4 U/mL) had %CVs ranging from 3.3% to 8.6%.

   The inter-assay reproducibility was determined by testing 6 samples in duplicate 8 times for 5 consecutive days. Two samples with high Anti-RNAP III concentrations (56.0 and 157.4 U/mL) had %CVs of 3.2% and 4.1 %, four samples close to the assay cut-off (17.2-33.8 U/mL) had %CVs ranging from 3.5% to 7.3%.

   The inter-lot reproducibility was determined by testing 6 samples in duplicate on two lots of plates seven times. Four low concentration sample (16.8-33.8 U/mL) had %CVs ranging from 2.6-4.6%. Two high concentration samples (58.8-153.6 U/mL) had %CVs ranging from 1.3-2.8%. The overall %CV ranged from 1.3-4.6%.
   b. *Linearity/assay reportable range:*
   There is no claim for linearity for this assay.
   The assay reportable range is from 5-200 U/mL.
   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   There is no reference standard for anti-RNAP III. The calibrators are prepared in-house and arbitrary units are assigned during development process. Positive and negative controls are not supplied but are recommended.
   d. *Detection limit:*

The lower limit of assay range was determined by dilution study. A sample that had 17 U/mL anti-RNAP III was chosen and serially diluted. The serial dilutions were measured and the criteria were:

1) CV (%) of eight repetitive assay is lower than 10
2) Mean ± 2SD of the dilution does not cross over with the adjacent dilution.

Results showed that 4.1 U/mL was the lowest value that satisfied the above conditions.

e. *Analytical specificity:*

Interference by endogenous substances: The package insert states that highly hemolyzed, icteric, lipemic, or microbially contaminated samples should not be used in these assays. Interference studies were performed on aliquots of five samples spiked with different concentrations of endogenous substances, namely hemoglobin, bilirubin C, bilirubin F, formazine (chyle) and rheumatoid factor (RF). The assay results were not affected with hemoglobin (up to 523 mg/dL), bilirubin C (up to 19.4 mg/dL), bilirubin F (up to 20.9 mg/dL), formazine (up to 2,800 units as) and RF (up to 500 IU/mL).

For assessing crossreactivity, the assay was tested on other autoimmune disease samples (MCTD, DM, PM, RA, SLE, and Vasculitis). Results showed minimal or no crossreactivity (refer to summary table below). Since Sjogrën's Syndrome (SjS) samples were not tested, the package insert states that the assay was not tested with Sjogrën's Syndrome (SjS) samples.
The assay was also tested on ten *E. coli* antibody positive samples and no crossreactivity was observed.

Disease	Positive sample	Positive rate
MCTD	2/21	9.5%
DM	1/21	4.8%
PM	1/23	4.3%
RA	0/25	0.0%
SLE	1/33	3.0%
Vasculitis	1/21	4.8%

f. *Assay cut-off:*
The cut-off value of 28 U/mL was established from a combined panel of 357 specimens collected from 143 healthy blood donors (HBD), 158 diagnosed SSc patients and 56 autoimmune diseases (33 SLE. 5 DM, 8 PM, 10 RA). The mean anti-RNAP III concentration of the HBD samples was 2.6 U/mL with a SD of 5.4 U/mL. Of the 143 HBD samples, only 2 samples had results above 20.5 U/mL, i.e. 42.1 and 42.4 U/mL. The anti-RNAP III concentrations for the non-SSc diseases were from 0.0-7.8 U/mL except for 1 SLE patient with a result of 52.7 U/mL.
75 of the 158 SSc samples were positive on the Anti-RNAP III ELISA kit with results ranging from 35.5-354.8 U/mL; and 83 of the 158 samples were negative with results ranging from 0.0-27.3 U/mL. An ROC analysis based on the 158 SSc samples and 199 normal plus target disease patients indicated a

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range between 27-38U/mL. The cut-off of 28 U/mL was selected based on the fact that the highest value of the negative SSc sample was 27.3 U/mL.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      Testing was performed on 211 samples which included 53 healthy blood donors and 158 Systemic sclerosis (SSc) samples. The positive percent agreement was 45.5% (76/167); the negative percent agreement was 97.7% (43/44); and the overall agreement was 56.4% (119/211). The low correlation is due to fact that the predicate ANA IFA method detects all antinuclear antibodies, while the Anti-RNAP III ELISA method detects antibodies only to RNAP III. Results are summarized below.

		Immuno Concepts ANA Hep-2000®Ro IFA		
		Positive	Negative	Total
MBL Anti-RNAP III ELISA	Positive	76	1	77
	Negative	91	43	134
	Total	167	44	211

Positive percent Agreement: 45.5% (76/167)
Negative percent agreement: 97.7% (43/44)
Overall percent agreement: 56.4% (119/211)

   b. *Matrix comparison:*
      Not applicable.

3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
      The MBL Anti-RNAP III clinical sensitivity and specificity study evaluated 609 clinically defined samples from patients with the following diagnosis: 257 Systemic sclerosis (SSc), 33 Systemic Lupus Erythematosus (SLE), 25 Rheumatoid Arthritis (RA), 23 Polymyositis (PM), 21 Dermatomyositis (DM), 21 Vasculitis, 21 Mixed Connective Tissue Disease (MCTD) and 208 healthy subjects. No pediatric samples were included in this study. Sensitivity was 23.4% (60/257) and the specificity was 97.4% (343/352) (see table below). The low sensitivity is due to the fact that the MBL Anti-RNAP III test only detects a subset of SSc patients.

		Diagnosis		
		Positive (Systemic Sclerosis)	Negative [Disease Controls: (MCTD, DM, PM, RA, SLE, Vasc) and Healthy Controls]	Total
MBL Anti-RNAP III	Positive	60	9	69
	Negative	197	343	540
	Total	257	352	609

Sensitivity: 23.4% (60/257) (95% C I: 18.31 – 29.0%)
Specificity: 97.4% (343/352) (95% C I: 95.2 – 98.8%)

The low sensitivity and high specificity of the SSc markers are supported by published literature as summarized in the following table.

Reported positivity of SSc Seromarkers:

Source Marker	Anti-centromere Ab	Anti-Th/To	Anti-U1RNP	Anti-PM-Scl	Anti-U3RNP	Anti-RNA Pol I or III	Anti-Scl-70 (Topo I)
Medsger, 1997 (1)	25%	5%	10%	5%	5%	20%	15%
Kuwana, et. Al. 1993 (3) [a]	-	-	-	-	-	5%	-
Okano, et. Al. 1993 (4)	20.6%	4.4%	11.1%[b]	3.2%	-	22.6%[c]	19.8%
Kuwana, et. Al. 1994 (6)[d]	18%	-	8%	3%	5%	24%	22%
Kuwana, et. Al. 1994 (6)[e]	11%	-	32%	0%	45%	14%	14%
Kuwana, et. Al. 2002 (11)	-	4.6%	-	-	-	-	-
Kuwana, et. Al. 2005 (13)	-	-	-	-	-	15%[f]	-

a) Japanese SSc patients (n=275)
b) U1, U2, U4/U6 and U5 RNP total
c) RNAP III only
d) North American Caucasian patients (n=358)
e) North American Black patients (n=22)
f) American patients (n=196, 134 white, 55 African American, 5 Hispanic and 2 Asian)

    b. Other clinical supportive data (when a is not applicable):
       Not applicable.
4. <u>Clinical cut-off</u>**:**
    Same as assay cut-off.
5. <u>Expected values/Reference range</u>:
    Expected values in the normal population should be 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.