510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042680

B. Purpose for Submission:
This is a new device.

C. Measurand:
Anti-nuclear Antibody (ANA)

D. Type of Test:
Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:
Corgenix, Inc.

F. Proprietary and Established Names:
RhiGene HEP-ANA Test System

G. Regulatory Information:
1. Regulation section:
   21 CFR§ 866.5100, Antinuclear Antibody, Immunological Test System
2. Classification:
   Class II
3. Product code:
   DHN - Antinuclear Antibody, Indirect Immunofluorescent, Antigen, Control
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   The RhiGene HEP-ANA Test System is an indirect fluorescent antibody assay using Hep-2 cells as a substrate for the qualitative and/or semi-quantitative determination of antinuclear antibodies in human serum. The RhiGene HEP-ANA Test System is intended for use as an aid in the diagnosis of certain autoimmune diseases.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   This device is for prescription use only.
4. Special instrument requirements:
   Fluorescent microscope

I. Device Description:

The RhiGene HEP-ANA Test System is an indirect immunofluorescent antibody assay. The assay components include the following:

- Substrate slides (forty foil wrapped slides of eight wells, with HEp-2 tissue culture sells fixed onto each well)
- Fluorescent antibody conjugate (contains 8.5 mL fluorescein isothiocyanate labeled goat antihuman immunoglobulin with 0.008% Evans Blue counterstain, 2% bovine serum albumin and 0.1% sodium azide)
- Positive control serum (Contains 0.5 mL of ANA positive human control serum ,homogeneous pattern, with 2% Bovine serum albumin and 0.1% sodium azide)
- Negative control serum (contains 0.5 mL of ANA negative human control serum, with 2% Bovine serum albumin and 0.1% sodium azide)
- Mounting medium (contains 3.5 mL phosphate buffered glycerol of pH 7.4 ±2
- Phosphate buffered saline (PBS)

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Bion Titer-Fluor ANA Test
2. Predicate 510(k) number(s):
   k872845
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	RhiGene HEP-ANA Test	**Titer-Fluor ANA Test**
Principle	Indirect immunofluorescence	Same
Intended Use	Determination of ANA in human serum to aid in the diagnosis of certain autoimmune diseases	Same
Type of test	Qualitative and/or semi-quantitative	Same
Sample matrix	Serum	Same
Substrate	HEp-2 cell	Same

Differences		
Item	Device	Predicate
Assay time	50 minutes at RT	80 minutes at RT
Conjugate	FITC goat anti-human immunoglobulins	FITC goat anti-human IgG (heavy chain and light chain)

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

L. Test Principle:
The RhiGene HEP-ANA Test System utilizes an indirect fluorescent antibody assay method. Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complex will not be formed and serum components will be washed away. Fluorescein labeled antihuman antibody is added to the reaction site which binds with the complexes formed .This results in a positive reaction of bright apple-green fluorescence. If no complexes are formed, the fluorescein antibody will be washed away, exhibiting a negative result.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*
      Intra-assay Reproducibility:
      Intra-assay reproducibility was determined by running three serum samples (low to moderate positive) using 3 replicates on 3 HEp-2 substrate slides from 3 different lots. Patterns and endpoint titers were determined. In the following tables, H = homogeneous pattern, S = Speckled pattern and N = Nucleolar pattern.

	Sample 1	Sample 2	Sample 3
Pattern	S	H	S
Frequency	9/9	9/9	9/9
Titer	1:640	1:160	1:40
Frequency	9/9	9/9	9/9

   Inter-assay Reproducibility:
   Inter-assay reproducibility was determined by running the same serum samples used above tested on 3 different days on 3 lots. Patterns and endpoint titers were determined.

	Sample 1	Sample 2	Sample 3
Pattern	S	H	S
Frequency	9/9	9/9	9/9
Titer	1:640	1:160	1:40
Frequency	9/9	9/9	9/9

   Inter-lot Reproducibility:
   Inter-lot reproducibility was determined by comparing value recovery of the serum samples on 3 different lots of HEP-ANA Test system.

	Lot 1	Lot 2	Lot 3
Sample 1	1:320 S+H	1:320 S+H	1:320 S+H
Sample 2	1:640 S	1:640 S	1:640 S

Sample 3	1:160 H	1:160 H	1:160 H

Intra-assay, intra-assay, and inter-lot studies showed 100% reproducibility.

b. *Linearity/assay reportable range:*
Not applicable for this type of application.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No recognized reference materials are available. Positive control is from BioMedical Resource (CA, USA) and Negative Control is from International Immunology Corporation (Japan). Other vendors might be used depending on reagent availability. The positive control should be more than 1+ positive at 1:64 dilution and the staining pattern must be Homogeneous or Speckled. The Negative control must be negative without dilution.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
<u>Interfering Substances:</u>
Different levels of hemoglobin, bilirubin, chyle, Rheumatoid Factor were added to patient specimens at various levels and tested using HEP-ANA Test System. The results indicated that the addition of Hemoglobin (up to 180 mg/dL), Bilirubin (up to 20.0 mg/dL), chyle (up to 2,780 units as Formazine) and Rheumatoid Factor (up to 520 IU/mL) have no effect on assay results.

f. *Assay cut-off:*
Assay cut-off of 1:40 was from a literature citation (Tan et. al, Arthritis Rheum. 40:1601-1611(1977). Results from sensitivity study (measured from 88 samples with SLE patients) and specificity study (measured from 271 healthy donors) also suggests 1:40 as a screening cut-off point.

2. <u>Comparison studies:</u>
a. *Method comparison with predicate device:*
The table below shows the results of comparison of serum samples (n=157) that were tested with the HEP-ANA Test System and the predicate device (Titer-Fluor ANA Test System). No demographics were provided for these samples. It was claimed that these samples were from a Japanese university hospital without any personal information.

		Titer-Fluor ANA		
		Positive	Negative	Total
HEP-ANA	Positive	54	1	55
Test	Negative	3	55	58
System	Total	57	56	113

Positive agreement: 94.7% (54/57)
Negative agreement: 98.2% (55/56)
Total agreement: 96.5% (109/113)

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

3. <u>Clinical studies:</u>
a. *Clinical Sensitivity:*
Fifty-six serum samples from a population of autoimmune disease patients

consisted of SLE (n=12), Sjogren's syndrome (SjS, n =13), systemic sclerosis (SSc, n=9), mixed connective tissue disease (MCTD, n=4), dermatomyositis (DM, n=7), polymyositis (PM, n=4) and Rheumatoid Arthritis (RA, n=7) were tested. Results showed 39 of the 56 samples were positive (69.6%). The following table shows sensitivity for each of the disease categories tested.

Disease	N	Positive	Negative	Clinical Sensitivity
SLE	12	11	1	91.7%
SjS	13	9	4	69.2%
SSc	9	7	2	77.8%
MCTD	4	4	0	100%
PM/DM	11	5	6	45.5%
RA	7	3	4	42.9%
Total	56	39	17	69.6%

b. *Clinical specificity:*
41 out of 50 healthy blood donor samples were negative on HEP-ANA Test System. Six of the nine false positive samples had a homogeneous pattern, two had a mixed homogeneous and speckled pattern and one with a speckled pattern. The ANA titers for these false positive samples ranged from 1:40 to 1:160. The clinical specificity was determined to be 82%.

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

4. <u>Clinical cut-off</u>**:**
See assay cut-off.

5. <u>Expected values/Reference range:</u>
The following is the incidence of antinuclear antibodies with HEP-ANA Test System in healthy blood donors and patient population. (Kavanaugh, A. et. al, Guidelines for Clinical Use of the Antinuclear Antibody Test ,Arch Pathol Lab Med.,124 71-81,2000).

Clinical Diagnosis	%Positive
Control	18%
SLE	92%
SjS	69%
SSc	78%
MCTD	100%
PM/DM	46%
RA	43%

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.