510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k131791

B. Purpose for Submission:

New Assay

C. Measurand:

Anti-nuclear antibodies (ANA)

D. Type of Test:

Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:

EUROIMMUN US INC.

F. Proprietary and Established Names:

EUROIMMUN IFA 40: HEp-20-10

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 EUROIMMUN IFA 40: HEp-20-10 is an indirect immunofluorescence antibody test for the qualitative or semi-quantitative detection of antibodies against cell nuclei (ANA) in human serum. This test system is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.

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: Equipped with a 488 nm excitation filter; 510 nm color separator; and 520 nm blocking filter with a 100 watt (W) mercury vapor lamp light source or LED blue light.

I. Device Description:

The test system consists of BIOCHIPs coated with HEp-20-10 cells. It includes a fluorescein-labeled goat anti-human IgG, a positive and negative control, salt for PBS, Tween 20, embedding medium, cover glasses and instruction booklet. Reagent trays for the TITERPLANE technique are required but ordered separately.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   ImmunoConcepts Hep-2000 ANA-Ro IFA, k972145

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Qualitative or semi-quantitative detection of antibodies against cell nuclei (ANA). The test system is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.	Same
Methodology	IFA	Same
Procedure	Standard IFA technique; serum incubation with cells, followed by a wash step, incubation with fluorescein–labelled anti–human globulin, wash step, embedding and reading fluorescence with a fluorescence microscope.	Same
Recommended Sample Dilution for Screen	1:40	Same
Cut-off Level	1:40	Same
Reported Results	Qualitative, Semi-quantitative Titer	Same
Sample Matrix	Serum	Same
Mounting Medium	Glycerol	Same

Similarities		
Item	Device	Predicate
Conjugate	FITC-labelled goat anti-human IgG	Same

Differences		
Item	Device	Predicate
Slide format/Antigen Source	BIOCHIP TITERPLANE / HEp-20-10 cells are bound to the BIOCHIPs	IFA slides/ HEp-2000 cells are bound to the test wells
Controls	1 Positive Control (homogenous pattern) 1 Negative Control	5 Positive controls (one each for the following patterns: SSA/Ro, homogeneous, speckled, nucleolar, centromere) 1 Titratable control serum 1 Negative control serum

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

Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510(k)) Submissions (January 22, 2009).

L. Test Principle:

Patient samples are diluted 1:40 in PBS-Tween, 30 µL of each diluted patient sample are added to each reaction field of the reagent tray. Reactions are started by fitting the BIOCHIP Slides containing the sections from the substrate (HEp-20-10 cells) into the corresponding recesses of the reagent tray and incubated for 30 minutes at room temperature. Specific antibodies attach to the HEp-20-10 antigens. After incubation the BIOCHIP Slides are washed with PBS-Tween to remove unbound antibodies. In the meantime, 25 µL of fluorescein–labelled anti–human globulin are added to each reaction field of a clean reagent tray and the BIOCHIP Slides placed into the recesses of the tray. After 30 minutes incubation at room temperature, the BIOCHIPs are again washed with PBS-Tween to remove any unbound fluorescein-labelled reagent. 10 µL of Embedding medium are placed for each reaction field on a cover glass and the BIOCHIP Slides, with the BIOCHIPs facing downwards, placed onto the prepared cover glass. Fluorescence is read with a fluorescence microscope.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

The fluorescence intensity level is the intensity of the specific fluorescence expressed as a numeric value. These values can vary from "0" (no specific fluorescence) to "4+" (strong specific fluorescence). The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	Interpretation
0	Negative: no specific fluorescence
1+	Positive: Weak visible reaction; dim subdued fluorescence
2+	Positive: Moderate visible reaction; green fluorescence
3+	Positive: Strong visible reaction; brilliant green fluorescence
4+	High Positive: Very strong visible reaction; brilliant green

Interpretation of test results: A serum dilution is considered negative for ANA antibodies if the cells exhibit < 1+ fluorescence and no discernible pattern. Cells appear reddish-orange due to the Evans Blue counterstain. Likewise, a serum dilution is considered positive for ANA antibodies if the cells exhibit ≥ 1+ fluorescence and a discernible pattern. A sample is considered positive for ANA antibodies if it exhibits exhibit ≥ 1+ fluorescence and a discernible pattern at a sample dilution of 1:40 or greater. Technicians should report all titers and patterns seen.

a. Precision/Reproducibility:

Reproducibility was investigated using a panel of serum samples representing the range of patterns at different intensities. Samples were manually diluted in two-fold steps from a starting dilution of 1:40.

Intra-Assay Reproducibility: Twenty-nine (29) samples with different patterns and titers were each tested 10 times according to the package insert and evaluated by the same technician. None of the readings between replicates differed by more than ± 1 fluorescence level; positive samples were not found negative (and vice versa) and the observed patterns did not change.

Inter-Assay Reproducibility: Twenty-nine (29) samples with different patterns and titers were each tested by 20 measurements obtained on 5 different days with 2 runs per day and 2 replicates (total 20 replicates) according to the package insert and evaluated by the same technician. None of the readings between replicates differed by more than ± 1 fluorescence level; positive samples were not found negative (and vice versa) and the observed patterns did not change.

Inter-Lot Reproducibility: Twenty-nine (29) samples with different patterns and titers were each tested by 6 measurements obtained by testing each sample in duplicate on three separate lots in three runs, 1 run per lot (total 6 replicates). The EUROIMMUN IFA 40: HEp-20-10 assays were processed according to the package insert and evaluated by the same technician. The results of the different lots did not exceed the fluorescence intensity of ± 1 intensity level, positive samples were not found negative and vice versa, and the observed patterns did not change.

Inter-Observer Reproducibility was determined by a separate independent reading of the Inter- Assay slides by a second technician for all 29 samples. The results of the different assays did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level, positive samples were not found negative and vice versa, and the observed patterns did not change. The Inter-Observer Reproducibility Study was performed in a US laboratory setting.

Semi-quantitative Reproducibility: Fourteen (14) positive samples and one negative sample were serially diluted from 1:40 to 1:10,240 then tested in duplicate in

four separate runs and read by two different observers (total 16 replicates). Three different lots were used in the experiment. Each of the seven main patterns (homogeneous, granular, nucleolar, centromeres, nuclear dots, nuclear membrane and cytoplasmic) were represented by a lower titer (1:80 – 1: 320) positive sample and a higher titer (1:1,280 – 1:10,240) positive sample. Fluorescence intensities ranged from 1+ to 4+. The results of the different assays for each dilution did not exceed the fluorescence intensity of ± 1 intensity level, so the endpoint titer did not deviate more than ± 1 titer level, and the observed patterns did not change.

b. Linearity/assay reportable range:

Six samples with different combinations of mixed staining patterns were serially diluted and tested with the EUROIMMUN IFA 40: HEp-20-10 assay. The samples were assayed according to the package insert. Each sample/dilution combination was tested and the fluorescence intensity and pattern were recorded.

Mixed patterns could be distinguished in every dilution, and the samples showed a decrease in fluorescence intensity as the samples were diluted. The pattern of the samples did not change with dilution. Acceptable deviation of fluorescence intensity: ± 1 intensity level.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability: A recognized standard or reference material for Anti-Nuclear Antibodies is not available.

Controls: Negative and positive controls are included in the kit. The negative control should exhibit less than 1+ or no specific fluorescence (autoantibody negative). The positive control should exhibit a homogenous staining pattern with a fluorescence intensity of >1+ (3 to 4+). The homogenous pattern of the positive control was confirmed via testing. EUROIMMUN Inc. recommends using the positive and negative controls as stated within the labeling (Instructions for Use).

Stability: Ongoing real-time stability tests of the kit are being conducted in accordance with *DIN EN 13640:2002: Stability testing of in vitro diagnostics reagents*. Three production lots of all kit reagents are tested. Current testing supports a stability claim of 12 months when stored at +2°C – +8°C.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Cross-reactivity: The specificity of the EUROIMMUN IFA 40: HEp-20-10 was verified using the ANA reference panel of the CDC (Centers for Disease Control and Prevention, Atlanta, USA). The CDC samples were assayed according to the package insert by the same technician at the same day. The results are in line with the characterization by the CDC with the exception of CDC sample No. 12. This sample was also tested using the EUROIMMUN ANA IFA: HEp-20-10 (K070763) and the ImmunoConcepts® ºHEp-2000 ANA-Ro IFA (K972145). The sample was found negative with all three test systems.

In addition to the CDC panel, clinical samples positive for ANCA-associated vasculitis, Crohn's disease, ulcerative colitis, celiac disease and infectious diseases (*Chlamydia pneumoniae* and Epstein-Barr virus) each 10 samples were investigated. The results do not indicate any cross reactivity.

Interfering substances: The effect of interfering substances on assay results were tested by spiking 20 clinical samples with hemoglobin (0, 250 & 500 mg/dL), bilirubin (0, 10 & 40 mg/dL) and triglycerides (0, 500 & 2000 mg/dL). The samples consisted of negative (< 1:40), weak positive and strong positive samples with varying pattern specificities. Interferences from human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) was tested by diluting these samples 1:1 with high positive HAMA serum or a high positive RF serum respectively. The spiked samples were incubated with the EUROIMMUN IFA 40: HEp-20-10 according to the package insert by the same technician on the same day. The deviation in fluorescence intensity level did not exceed ± 1. The results indicated that hemoglobin (up to 1000 mg/dL), bilirubin (up to 40 mg/dL), triglyceride (up to 2000 mg/dL), HAMA and RF at the concentrations indicated have no effect on assay results. No interference was observed.

f. Assay cut-off:

The recommended starting dilution, above which the result is reported as positive and below which the result is reported as negative, is 1:40. The manufacturer suggests performing four-fold dilutions but recommends that each laboratory establish its own titering protocol. The titers of 1:40 and 1:80 are considered low titers, 1:160 and 1:320 are considered medium titers, and 1:640 and greater are considered high titers. Assay cut-off of 1:40 was determined from the literature (*Range of antinuclear antibodies in "healthy" individuals*, Arthritis & Rheumatism 40, 1997; *Guidelines for Clinical Use of the Antinuclear Antibody Test and Tests for Specific Autoantibodies to Nuclear Antigens*, Arch Pathol Lab Med. 124, 2000).

2. Comparison studies:

a. Method comparison with predicate device:

Qualitative: A comparison study of patient samples sent for routine ANA testing at an academic research center was performed using a cohort of 200 blinded prospective samples where age and sex information were available. The samples were tested concurrently with EUROIMMUN HEp-20-10 IFA 40 and the predicate ImmunoConcepts HEp-2000 ANA-Ro IFA. The panel consisted of 59 men and 141 women; patient age ranged from 19 to 89 years with an average age of 52 years. The tests were performed (1:40 dilution) in single determinations.

		ImmunoConcepts HEp-2000 ANA-Ro IFA		
		Positive	Negative	Total
EUROIMMUN IFA 40:HEp-20-10	Positive	61	6	67
	Negative	5	128	133
	Total	66	134	200

% Positive Agreement 61/66 = 92.4% 95% CI: 83.2% - 97.5%

% Negative Agreement 128/134 = 95.5% 95% CI: 90.5% - 98.3%

Semi-Quantitative/Quantitative: A comparison study of reciprocal titers and patterns was performed using a different cohort of 156 prospective blinded samples from patients sent for routine ANA screening. The samples were tested with EUROIMMUN HEp-20-10 IFA 40 concurrently with the ImmunoConcepts® HEp-2000 ANA-Ro IFA predicate. The panel consisted 62 men and 94 women, average age of 52 years, age ranged from 19 to 90 years. The tests were performed according to the package insert in single determinations.

		Predicate		
		Positive	Negative	Total
EUROIMMUN IFA 40:HEp-20-10	Positive	110	0	110
	Negative	0	46	46
	Total	110	46	156

Positive % Agreement = 110/110 = 100% (95% CI: 96.7% ─ 100%)
Negative % Agreement = 46/46 = 100% (95% CI: 92.3% ─ 100%)

Pattern agreement is tabulated below. Some samples had more than one pattern; each was reported individually; overall agreement was 91.4%.

		Observed Pattern		
Pattern	n	Predicate	EUROIMMUN	% Agreement
Homogenous	41	41	41	100
Granular/Speckled	28	26	28	92.9
Nucleolar	24	23	22	87.5
Centromere	15	15	15	100
Nuclear Dot	9	9	9	100
Nuclear Membrane	3	2	3	67
Cytoplasmic	28	28	26	93

Comparison of reciprocal titers between the predicate and the new assay is shown below. If multiple patterns were reported, those titers are reported separately:

		Predicate: reciprocal titer										
		<40	40	80	160	320	640	1280	2560	5120	10240	Total
EUROIMMUN	<40	46		1	2							49
	40			2								2
	80		7	7	4							18
	160	2		18	13	7		2*				42
	320	2			16	14	3		1*			36
	640					9	5	2	1*			17
	1280						4	6	3			13
	2560							5	2	1	2*	10
	5120								3			3
	10240									1	2	3
	Total	50	7	28	35	30	12	15	10	2	4	**193**

*HEp-2000 Fluorescent ANA-Ro Test System overexpresses the SS-A/Ro autoantigen in the HEp-2000® cells; samples that contain anti-Ro/SS-A antibodies may show higher titer values on these cells than the values obtained on non-transfected HEp-2 cells. A distinct speckled and nucleolar pattern is seen in 10 - 20% of the interphase nuclei; some cells may show staining in the cytoplasm.

b. 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):

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

A panel of 200 sera from normal healthy adult blood donors of mixed age and gender (155 men, 45 women, mean age 38 years, age range 18-68 years) was tested with the EUROIMMUN IFA 40: HEp-20-10 kit to establish of the assay. The samples were assayed according to the package insert on the same day by three technicians. Prevalence, defined as titers of >1:40, was found about 3.5%. As per the American College of Rheumatology a "prevalence of ANAs in healthy individuals is about 3.0 – 15.0%".

In a separate study, 138 samples with age and sex data available sent for ANA antibody testing were collected in sequential order for two days. The panel consisted of samples from 53 men and 85 women submitted for a routine health screening. Age ranged from 0 to 89 years with an average age of 51 years. Testing, at initial dilution of 1:40, was performed. Sixteen (16) of the 138 samples were positive for anti-nuclear antibodies using the EUROIMMUN IFA 40: HEp-20-10 kit, resulting in a prevalence of 11.6% (95% C.I.: 6.8% – 18.6%). Clinical diagnosis was not available.

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.