

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

A. 510(k) Number:

k070763

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:

EUROIMMUN US, LLC.

F. Proprietary and Established Names:

EUROIMMUNE ANA IFA: 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:

(82) Immunology

H. Intended Use:

1. Intended use(s):

This is an indirect immunofluorescent antibody test for the qualitative or semi-quantitative detection of antinuclear antibodies (ANA) in human serum and EDTA plasma. This test is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

The device is for prescription use only.

4. Special instrument requirements:

Fluorescent microscope with a 488 nm excitation filter, 510 nm color separator and 520 nm blocking filter with a 100 W mercury vapor lamp light source or LED 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):

ImmunoConcepts Hep-2000 Fluorescent ANA-Ro Test System

2. Predicate 510(k) number(s):
k972145
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EUROIMMUNE ANA IFA: Hep-20-10	ImmunoConcepts Hep-2000 Fluorescent ANA-Ro Test System
Intended Use	Detection of anti-nuclear antibodies (ANA)	Same
Technology	Immunofluorescence	Same
Differences		
Item	Device	Predicate
Sample	Serum or plasma	Serum
Technology	BIOCHIP TITERPLANE technology (cleared under k051489) Hep-20-10 cells are bound to the BIOCHIPS	Standard IFA technology Hep-2000 cells are bound to the test wells
Controls	1 positive control 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
Type of test	Qualitative or Semi-quantitative	Semi-quantitative
Substrate	Hep-20-10 cells	Hep-2 cells
Conjugate	FITC goat anti-human immunoglobulins	FITC goat anti-human IgG (heavy and light chains)
Cut-off level	1:100 dilution	1:40 dilution

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

None referenced.

L. Test Principle:

The procedure follows the TITERPLANE technique. Patient samples, controls and in separate steps conjugate and embedding medium are applied to the reaction fields of a reaction tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPS of the slide come in contact with fluids, and the individual reactions commence simultaneously. The fluids are confined to the recessed wells eliminating the need to use a conventional “humidity chamber”.

Patient samples are diluted 1:100 in PBS-Tween, 25 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, 20 μ L of fluorescein-labeled 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 minute incubation at room temperature, the BIOCHIPS are again washed with PBS-Tween to remove any unbound fluorescein-labeled reagent. Ten (10) μ L 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:

a. *Precision/Reproducibility:*

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 “5” (extreme strong specific fluorescence). The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	Evaluation
0	no specific fluorescence
1	positive reaction, very weak specific reaction visible
2	positive reaction, weak specific reaction visible
3	positive reaction, specific reaction well visible
4	positive reaction, strong specific reaction visible
5	positive reaction, very strong specific reaction visible

Intra-assay reproducibility was determined by 10 fold repeated measurements of 9 dilutions of a characterized positive serum sample and a negative sample. The results did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level.

Inter-assay reproducibility was determined by repeated measurements of a characterized serum sample in 9 dilutions and a negative sample at 5 different times. 2 slides were tested with each run. The results did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level.

Lot to Lot reproducibility was determined by measurements of 9 dilutions of a characterized serum sample and a negative sample using different lots within a period of over two years (2001 to 2002). Kit and component lot number are the same. Two to thirteen (2 to 13) slides were tested with each lot. Tests were evaluated by the same technician. The results did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level. Pattern descriptions of samples used on the study were indicated.

Inter-observer reproducibility was determined by 10 fold repeated determination of several dilutions of a positive serum sample and a negative serum sample. The tests were processed and then intensity levels read by three different observers. The whole range of fluorescence intensity levels (0 to 5) is covered by the study. The results obtained by the three observers did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level.

- 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.

Negative and positive controls are included. The positive control is ready for use with a 3-4+ fluorescence with a homogeneous staining pattern. Negative control is ready for use and is auto-antibody negative.

A stability study was performed using test kits at 4°C for day 0, 12 months, 24 months and 36 months. Test kits characterized controls and reference sera (stored at -70°C), positive for different patterns (nDNS, AMA-M2, Actin, HU and RANA) were determined with these test kits. The tests were done on three different kit lots. The results, compared to those of day 0, did not exceed the acceptable deviation of fluorescence intensity of ± 1 intensity level up to a period of 36 months.

- d. *Detection limit:*
Not applicable
- e. *Analytical specificity:*

Cross-reactivity

Cross reactivity was determined using 11 characterized sera of patients with different diseases obtained from CDC. These sera showed the characteristic fluorescence patterns expected for the related diseases and did not indicate any cross-reactivity to ANA.

Interfering substances

Different levels of hemoglobin, bilirubin, chyle, and rheumatoid factor were added to patient specimens at various levels and tested using the ANA IFA: Hep-20-10 test system. The results indicated that the addition of hemoglobin (up to 500 mg/dL), bilirubin (Up to 40 mg/dL), triglyceride (Up to 2000 mg/dL) and rheumatoid factor (up to 200 U/mL) have no effect on assay results.

- f. *Assay cut-off:*
The dilution starting point is 1:100. This was determined during the pre-production product development stage based on the evaluation of clinically defined donor samples yielding the appropriate clinical sensitivity and

specificity meeting the input criteria based on the current literature and historical experience. The cut off dilution was verified using a total of 398 sera, including 198 prospective samples and 200 normal samples. Of the 200 normals, 90% were negative using the cut-off starting point of 1:100. This is in line with prevalence reported in the literature and with the pre-production input criteria. Samples can be further diluted by a factor of 10. There is no upper limit to the measurement range.

2. Comparison studies:

a. *Method comparison with predicate device:*

Comparison study between the EUROIMMUNE ANA IFA: Hep-20-10 and the predicate device, Immunoconcepts HEP-2000 Fluorescent ANA-Ro was performed on 198 prospective samples. The samples were from 58 men and 138 women and 2 with unknown gender. Age of subjects ranged from 0 to 91 years with an average age of 50.5 years. Age and sex had no significant influence on the test results. The results are summarized in the table below:

n = 198		Immunoconcepts HEP-2000. ANA-Ro IFA	
		positive	negative
EUROIMMUNE ANA IFA: Hep- 20-10	positive	135	0
	negative	4	59
	total	139	59

Total agreement = 194/198 = 98.0%

Positive % agreement = 135/139 = 97.0%

Negative% agreement = 59/59 = 100%

The four discrepant samples tested were prospective routine samples that were run in the laboratory on both assays. The labs routine method is the Immunoconcepts test, but the results are not reported based on the fluorescence intensities. In this laboratory the results are routinely converted to IU/ml against the dsDNA WHO standard using an automated reading program, validated by the laboratory. Although the samples resulted in a 1+ titer when read conventionally, the converted results were below 7.5 IU/ml and the samples were reported as negative. Therefore, the samples were not tested further. The IU/ml results are shown in the table below:

No.	Sample-ID	Sex	Age	Immunoconcepts		EUROIMMUN	Pattern
				fluorescence intensity	converted IU/ml (dsDNA WHO std.)	Result	
39	006-4186360	F	34 y	1+	6.7	neg	
81	006-4173489	M	25 y	1+	5.5	neg	
88	006-4172378	F	56 y	1+	5.6	neg	mitochondrial
102	006-4170215	F	18 y	1+	4.9	neg	

To determine the agreement of pattern evaluation, 111 ANA positive samples were tested on both devices. The samples were from 13 men, 79 women and 19 unknowns. Age ranged from 8 to 83 years with an average age of 53.7 years. The results are shown in the table below.

Pattern	n	Immunoconcepts HEP-2000 ANA-Ro IFA	EUROIMMUNE ANA IFA: Hep-20-10
Homogeneous	40	40	40
Speckled	24	24	24
Nucleolar	2	2	2
Centromere	26	26	26
Rim	19	19	19

Overall agreement = 100%

b. Matrix comparison:

Nineteen matched serum and EDTA plasma samples were used in the study. There was no deviation in fluorescence intensity of the serum and EDTA plasma samples.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

Eleven (11) characterized sera of patients with different diseases obtained from CDC were tested using the EUROIMMUNE ANA IFA: Hep-20-10 kit. The sera showed the characteristic patterns and endpoint titers expected for the related diseases.

In addition, five samples with different combinations of mixed staining patterns were evaluated with ANA-IFA: Hep-20-10 kit in 5 different dilutions. Mixed patterns could be distinguished in every dilution. The results are shown in the table below:

Sample-ID	Dilution				
	1:100	1:320	1:1000	1:3200	1:10000
070411-50	ANA granular 3 ANA nucleolar 2	ANA granular 3 ANA nucleolar 3	ANA granular 2 ANA nucleolar 3	ANA granular 1 ANA nucleolar 2	ANA nucleolar 1
070412-14	ANA homogeneous 4 ANA granular 3	ANA homogeneous 3 ANA granular 3	ANA homogeneous 2 ANA granular 2	negative	negative
070417-18	ANA homogeneous 3 ANA nucleolar 3	ANA homogeneous 3 ANA nucleolar 3	ANA homogeneous 2 ANA nucleolar 3	ANA homogeneous 1 ANA nucleolar 2	ANA homogeneous 1 ANA nucleolar 1
070416-14	ANA homogeneous 3 ANA nucleolar 1	ANA homogeneous 2	negative	negative	negative
070416-191	ANA granular 3 ANA nucleolar 2	ANA granular 3 ANA nucleolar 1	ANA granular 3 ANA nucleolar 1	ANA granular 2 ANA nucleolar 2	ANA granular 1 ANA nucleolar 1

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

A panel of 200 sera from normal healthy adult blood donors of mixed age and gender were tested to determine reference range. Prevalence of ANA was found to be about 10%. A prevalence of 6.9-25% was reported in literatures*. The reference range was determine as titer 1:<100.

*1. Forslid, J. Heigl, et al. The prevalence of antinuclear antibodies in healthy young persons and adults, comparing rat liver tissue sections with Hep-2 cells ab antigen substrate. Clin. Exp. Rheumatol.; Mar-Apr; 12(2): 137-41 (1994).

*2. Teubner, A. et al. Prevalence of circulating autoantibodies in health individuals. Med Klin.; Nov. 15; 97(11); 645-9 (2002).

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.