

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

A. 510(k) Number:

k072149

B. Purpose for Submission:

New device

C. Measurand:

Antinuclear IgG antibodies: Sm, RNP, SS-A, SS-B, Centromere B, Scl-70, Jo-1

D. Type of Test:

Qualitative, ELISA

E. Applicant:

PHADIA US Inc.

F. Proprietary and Established Names:

EliA Symphony Immunoassay for ImmunoCAP 100

EliA Symphony Immunoassay for ImmunoCAP 250

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5100, Antinuclear Antibody Immunological Test System

2. Classification:

Class II

3. Product codes:

LLL, Extractable Antinuclear Antibody, Antigen, Control

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

EliA Symphony for ImmunoCAP 100 is intended for the in vitro qualitative measurement of antinuclear IgG antibodies in human serum and plasma (heparin, EDTA and citrate). EliA Symphony is based on human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1 proteins and native purified Sm proteins as antigen and is useful as an aid in the clinical diagnosis of patients with systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and polymyositis/dermatomyositis, in conjunction with other laboratory and clinical findings. EliA Symphony uses the EliA IgG method on the instrument ImmunoCAP 100.

EliA Symphony for ImmunoCAP 250 is intended for the in vitro qualitative measurement of antinuclear IgG antibodies in human serum and plasma (heparin, EDTA and citrate). EliA Symphony is based on human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1 proteins and native purified Sm proteins as antigen and is useful as an aid in the clinical diagnosis of patients with systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and

polymyositis/dermatomyositis, in conjunction with other laboratory and clinical findings. EliA Symphony uses the EliA IgG method on the instrument ImmunoCAP 250.

EliA ANA Control is intended for laboratory use in monitoring the performance of in vitro measurement of antinuclear antibodies (ANA) with ImmunoCap 100 or ImmunoCap 250 using the EliA IgG method.

2. Indication(s) for use:
Same as Intended use.
3. Special conditions for use statement(s):
For prescription only.
4. Special instrument requirements:
ImmunoCAP 100
ImmunoCAP 250

I. Device Description:

The EliA reagents are available as modular packages, each purchased separately. The EliA Symphony wells are coated with human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1 and native purified Sm proteins. All packages except for the EliA ANA Control are required to carry out an EliA Symphony Test. The EliA Symphony wells are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant. The EliA Method-Specific reagents for ImmunoCap 100 or ImmunoCap 250 consists of: six levels of ready to use EliA IgG calibrators (0, 4, 10, 20, 100, 600 µg/L); IgG calibrator well (coated with mouse monoclonal antibodies); ready-to-use positive and negative controls; ready-to-use IgG curve control (20 µg/L); IgG conjugate (β-Galactosidase anti-IgG mouse monoclonal antibodies) in PBS; ready-to-use sample diluent (PBS with BSA). The EliA General reagents consist of: ready-to-use development solution (0.1% 4-Methylumbelliferyl-β-D-galactoside); ready-to-use stop solution (4% Sodium Carbonate); ready-to-use 96-MicroWell™ plates; and ImmunoCap washing solution

J. Substantial Equivalence Information:

1. Predicate device name(s):
INOVA Quanta Lite™ ENA 6
INOVA Nova Lite™ HEp-2
2. Predicate K number(s):
k961913 (ENA 6)
k880736 (HEp-2 Centromere B)
3. Comparison with predicate:

Similarities			
Item	New Device	Predicate Devices	
	EliA Symphony Immunoassay for ImmunoCAP 100 and ImmunoCAP 250	QuantaLite™ ENA 6	Nova Lite™ HEp-2
Analyte detection	Antinuclear antibodies	Same	Same

Differences			
Item	New Device	Predicate Devices	
	EliA Symphony Immunoassay for ImmunoCAP 100 and ImmunoCAP 250	QuantaLite™ ENA 6	Nova Lite™ HEp-2
Intended Use	<p>For qualitative measurement of antinuclear IgG antibodies in human serum and plasma (heparin, EDTA and citrate) EliA Symphony is based on human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1 proteins and native purified Sm proteins as antigen and is useful as an aid in the clinical diagnosis of patients with systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and polymyositis/dermatomyositis, in conjunction with other laboratory and clinical findings. EliA Symphony uses the EliA IgG method on the instrument ImmunoCAP 100 or ImmunoCAP 250.</p>	<p>An enzyme-linked immunosorbent assay (ELISA) for semiquantitative detection of Sm, RNP, SS-A (60kDa, 52kDa) SS-B, Scl-70, and Jo-1 antibodies in human serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of SLE and related CTD such as Sjögren's syndrome.</p>	<p>An indirect immunofluorescent assay for the screening and semi-quantitative determination of anti-nuclear antibodies (ANA) in human serum. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of SLE or other connective tissue or rheumatic diseases.</p>
Antigen	Human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60kDa),	Purified RNP, SS-A, SS-B, Scl-70, Jo-1, Sm	Human epithelial cells (for centromere staining pattern)

	SS-B/La, Scl-70, Jo-1, Centromere B and native purified Sm proteins		
Test type	Qualitative	Semi-quantitative	Qualitative and semi-quantitative
Assay type	ELISA	ELISA	IFA
Concept	Modular reagents (test-method specific and general reagents)	Single kit	Single kit
Sample matrix	Serum and plasma (heparin, EDTA and citrate)	Serum	Serum
Assay platform	Solid phase 48 microwells	Solid phase 96 microwells	12 well Hep-2 slides
Calibrator	Liquid; 1.0 mL each/ 5 levels	Not applicable	Not applicable
Positive/ Negative Controls	Packaged and sold separately	Included in the kit	Included in the kit
Conjugate	β -Galactosidase anti-human IgG (mouse monoclonal antibodies)	Horse-radish peroxidase goat anti-human IgG	FITC goat Anti-human IgG
Signal	Optical density	Optical density	Fluorescence
Instrument	ImmunoCAP 100 and ImmunoCAP 250 (fully automated analyzers)	Microplate reader with 450 nm filter (and 620 nm for dual wavelength)	Fluorescence microscope with 495 nm exciter and 515 nm barrier filter
Reaction temperature	37°C controlled	Room temperature (20-26°C)	Room temperature (20-26°C)
Unit of measure	Ratio	Units	Titer and Nuclear staining Pattern
Result Interpretation	Negative: <0.7 Ratio Equivocal: 0.7 – 1.0 Ratio Positive: >1.0 Ratio	Negative: <20 Units Weak Positive: 20-39 Moderate Positive: 40-80 Strong Positive: >80	Neg: Specific staining \leq IFA Neg Control Pos: Specific staining > IFA Neg Control <u>Titer</u> : initial 1:40 dilution to endpoint Nuclear staining <u>Patterns</u> : Homogeneous, Peripheral, Speckled, Nucleolar, Centromere, Mitochondrial

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

None provided.

L. Test Principle:

The EliA Symphony Wells are coated with human recombinant U1RNP (RNP 70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70 and Jo-1 proteins, native purified Sm proteins. If present in the patient's specimen, antibodies to the antigens bind to their specific antigen. After washing away unbound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

M. Performance Characteristics (if/when applicable):**1. Analytical performance:****a. Precision/Reproducibility:**

The precision of the assay on ImmunoCAP 100 instrument was determined by testing four serum samples in two replicates on three instruments in 18 runs with a calibration curve in each run. Results showed that three positive samples with Ratio ranges from 1.5-12.8 had 3.3 - 5.4%CV and 3.6 – 3.9 % CV for the intra-assay and inter-assay studies respectively; and one negative sample with 0.7 Ratio had 5.6 %CV and 1.4% CV for the intra-assay and inter-assay studies respectively (see below).

ImmunoCAP 100			
Sample	Mean (Ratio)	Intra-assay (%CV)	Inter-assay (%CV)
1	0.7	5.6	1.4
2	1.5	4.7	3.7
3	5.9	3.3	3.9
4	12.8	5.4	3.6

The precision of the assay on ImmunoCAP 250 instrument was determined by testing four serum samples in duplicate on three instruments in 21 runs with a calibration curve in each run. Results showed that two positive samples with 2.3 – 11.9 Ratio ranges had 3.7 – 4.1%CV and 3.9 – 4.8 % CV for the intra-assay and inter-assay studies respectively; and two negative sample with 0.3 – 0.9 Ratio ranges had 8.8 – 14.8 %CV and 0.0 – 2.5% CV for the intra-assay and inter-assay studies respectively (see below).

ImmunoCAP 250			
Sample	Mean (Ratio)	Intra-assay (%CV)	Inter-assay (%CV)
1	0.3	14.8	2.5
2	0.9	8.8	0.0
3	2.3	4.1	3.9
4	11.9	3.7	4.8

b. *Linearity/assay reportable range:*

Hook Effect:

Hook effect was analyzed using dilutions from a high positive serum sample mix with an estimated concentration greater than the highest calibrator, Calibrator 600 (600 µ/L) in two runs with four replicates/sample. A complete calibration curve was included (2 replicates/ calibrator). No hook effect was observed for concentrations up to 6000 µ/L, which is 10 fold above the highest calibrator measuring range.

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

The EliA IgG calibrators are traceable to the International Reference Preparation (IRP) 67/86 of the Human Serum Immunoglobulins A, G, and M from WHO. New batches of IgG calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration (5 levels).

There are no international standards for ANA antibodies. The positive and negative controls are prepared in house and arbitrary Ratio units are assigned during the development process.

Stability studies were performed with the acceptance criteria of >90% recovery from date of manufacture. Stability data showed shelf life of 24 months for EliA Symphony wells and EliA ANA Controls. The expiration date claims are 18 months for EliA Symphony wells and 24 months for EliA ANA Controls.

d. *Detection limit:*

The lower limit of the measuring range was determined by measuring dilutions (1/2, 1/4, and 1/8) of Calibrator 4.0 (4 µg/L of IgG) in EliA sample diluent and run on EliA IgG Calibrator wells. The results in Response Units (RU) were compared with the result of the sample diluent on the different EliA Symphony wells. All samples were measured in duplicates. The discrimination ability 'D' of the assay should be >2 for calibrator 4 diluted 1:4 (i.e. 1.0 µg/L IgG/L). The 1/8 diluted calibrator 4 (0.5 µg/L) still can be discriminated from background given by the signal of the diluent on EliA Symphony wells (as shown in the table below). The lower limit of detection was set at 0.5 µg/L corresponding to 0.5 Ratio.

Results on Calibrator Wells			
Sample ID	Mean Response Units [RU]	SD [RU]	D
D. Cal 4 1:2	272	11.7	22.8
D. Cal 4 1:4	209	3.5	58.0
D. Cal 4 1:8	126	3.1	38.2
Results on Symphony Wells			
Sample Diluent	5	0.5	

e. *Analytical specificity:*

Interfering substances

The potential interferent and the corresponding blanks were added to the EliA ANA positive control used as a positive serum sample, diluted twice (1:2 and 1:4) with EliA diluent. The spiked samples (900 µL diluted serum and 10 µL of interferent) were tested in triplicate. The ratio of the result of the sample spiked with the interfering substances and the sample spiked with a buffer blank was determined as shown in the table below:

		Positive Sample			Equivocal Sample		
Additive	blank/spiked sample	Conc. [Ratio]	CV %	Ratio	Conc. [Ratio]	CV %	Ratio
Bilirubin F	blank	11.5	2.6	0.95	0.82	9.5	1.01
	sample	10.9	3.2		0.82	6.4	
Bilirubin C	blank	11.6	2.3	0.94	0.83	7.4	0.93
	sample	10.9	2.5		0.77	3.6	
Haemoglobin	blank	12.0	1.2	0.98	0.85	4.4	0.95
	sample	11.7	1.6		0.81	1.5	
Chyle	blank	11.4	9.4	1.08	0.83	3.8	1.05
	sample	12.3	1.4		0.87	6.7	
Rheumatoid factor	blank	12.4	3.0	0.97	0.82	1.4	0.97
	sample	12.0	4.3		0.80	3.3	

The interfering substances Bilirubin C (up to 0.211 mg/dL), Bilirubin F (up to 0.266 mg/dL), Chyle (up to 1570 Units/dL), Hemoglobin (up to 5.19 mg/dL) and Rheumatoid Factor (up to 5.0 IU/mL) did not adversely affect the results of the new device. The package insert states not to use lipemic, hemolyzed, icteric or microbially contaminated samples.

Crossreactivity with other autoantibodies: The EliA Symphony Immunoassay was tested with 17 sera containing other autoantibodies specific for tissue transglutaminase IgG (3), gliadin IgG (3), MPO ANCA (3), PR3 ANCA (3), thyroid globulin (3), and thyroid peroxidase (2). Results were all negative < 0.7 Ratio (ranged from 0.1 – 0.4 Ratio).

f. Assay cut-off:

The purpose of the normal sera studies was to evaluate expected values in the normal population and to confirm the defined cut-off. Samples from 400 apparently healthy Caucasian adult blood donors were measured. The individuals were equally distributed by age and gender. The results were equally distributed and not dependent on age or gender. The 95th percentile (0.4 Ratio) lies below the lower limit of the equivocal range (0.7-1.0 Ratio).

2. Comparison studies:

a. Method comparison with predicate device:

Comparative study on RNP, SS-A, SS-B, Scl-70, Jo-1, and native purified Sm proteins: testing was performed on 137 samples from clinically defined patients (30 SLE; 19 Sjögren's syndrome; 14 MCTD; 10 scleroderma; 12

myositis; 2 overlap syndrome) and 50 healthy patients on the EliA Symphony and the predicate device, QuantaLite ENA 6 Screening Test. Equivocals were excluded from the percent agreement calculations. The Positive Percent Agreement was 100% (82/82); the Negative Percent Agreement was 100% (55/55); and the Overall Agreement was 100% (137/137) (see table below).

		QuantaLite™ ENA 6 screening test		
		Positive	Negative	Total
EliA Symphony	Positive	82	0	82
	Negative	0	55	55
	Total	82	55	137

Comparative study on Centromere: testing was performed on 14 samples from clinically defined patients (5 CREST Syndrome, 1 SLE/CREST, 1 SLE and 7 healthy subjects) on the EliA Symphony and the predicate device, IIF HEp-2. The Positive Percent Agreement was 100% (7/7); the Negative Percent Agreement was 100% (7/7); and the Overall Agreement was 100% (14/14) (see table below).

		IIF HEp-2		
		Positive	Negative	Total
EliA Symphony	Positive	7	0	7
	Negative	0	7	7
	Total	7	7	14

Instrument platform comparison:

For this comparison study, a total of 36 samples (2 negative and 34 positive samples) distributed over the Ratio range were tested. All samples were run on three IC 250 instruments and three IC 100 instruments in two runs and single replicates. Regression analysis showed a slope of 0.9888 and a y-intercept of 0.0403 with $r = 0.989$. Specifications was fulfilled ($r > 0.9$).

b. Matrix comparison:

Serum and plasma (heparin, EDTA, citrate) samples, covering the Ratio range (0.1 – 5.4 Ratio) were compared to determine if any significant bias existed between matrices. The correlation coefficients were acceptable and no bias observed.

	N	Regression equation	r
Heparin	50	$y = 0.9970x - 0.0102$	0.9970
EDTA	50	$y = 1.0175x - 0.0249$	1.0180
Citrate	50	$y = 1.0379x - 0.0393$	1.0380

3. Clinical studies:

- a. *Clinical Sensitivity and specificity:*
The clinical sensitivity and specificity studies were evaluated using the same 137 clinical samples from the method comparison studies: 30 SLE; 19 Sjögren's syndrome; 14 MCTD; 10 scleroderma; 12 myositis; 2 overlap syndrome; 5 CREST Syndrome, 1 SLE/CREST, 1 SLE, and 57 healthy patients. All autoimmune disease patients were positive and all healthy subjects were negative with the new device. Clinical sensitivity and specificity of the new device are 100%.
 - b. Other clinical supportive data (when a. is not applicable):
Not applicable.
 - 4. Clinical cut-off:
Same as assay cut-off
 - 5. Expected values/Reference range:
Not applicable.
- 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.