

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

A. 510(k) Number:

k072393

B. Purpose for Submission:

New device

C. Measurand:

Anti-DNA antibodies

D. Type of Test:

Semi-quantitative fluoroenzyme immunoassay

E. Applicant:

Phadia US, Inc.

F. Proprietary and Established Names:

EliA™ dsDNA Immunoassay

EliA™ ANA Control

G. Regulatory Information:

1. Regulation section:

21 CFR§ 866.5100, Antinuclear Antibody Immunological Test System

21 CFR§ 862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

Device-Class II

Quality control material-Class I

3. Product code:

LSW, Anti-DNA Antibody, Antigen and Control

JJY, Multi-Analyte Controls (Assayed and Unassayed)

4. Panel:

(82) Immunology

(75) Chemistry

H. Intended Use:

1. Intended use(s):

EliA™ dsDNA is intended for the in vitro quantitative measurement of IgG antibodies directed to dsDNA in human serum and plasma (heparin, EDTA, citrate) as an aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA™ dsDNA uses the EliA IgG method on the instrument ImmunoCAP 100 and 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 above

3. Special conditions for use statement(s):

The device is for prescription use only.

4. Special instrument requirements:

ImmunoCAP 100 and ImmunoCAP 250 (k061165)

I. Device Description:

The EliA reagents are available as modular packages, each purchased separately. The EliA dsDNA wells are coated with double stranded plasmid DNA. These are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant. The EliA Method-Specific reagents consists of (1) sample diluent concentrate, (2) IgG Conjugate (blue colored) β -Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS, (3) ready-to-use 6 level IgG calibrators (human IgG concentrations of 0,4, 10, 20, 100 and 600 $\mu\text{g/L}$), (4) ready-to-use IgG Curve Control (20 $\mu\text{g/L}$), (5) IgG Calibrator well coated with mouse monoclonal antibody, (6) ready for use development solution containing 0.1% 4-methylumbelliferyl – β -D galactoside and (7) 4% sodium carbonate stop solution.

Curve Controls have defined ranges to check whether the stored calibration curve is still valid. Limits for the response of the Curve Controls are defined in the ImmunoCAP 100/250 Operator and Panel Software.

The EliA ANA is a two- level control (negative and positive). This is a mutiparameter control containing antibodies to dsDNA (k072393), RNP, Sm, Ro, La, Scl-70, CENP and Jo-1 (k072149 EliA Symphony ANA). The EliA ANA Control is prediluted and ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
DPC Anti-DNA
2. Predicate 510(k) number(s):
k874873
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EliA™ dsDNA	DPC Anti-DNA
Indications for Use	As an aid in the clinical diagnosis of systemic erythematosus (SLE)	Same
Calibration	Calibrated against the 1 st International Standard for anti-double stranded DNA coded Wo/80. Results are given in International Units (IU/mL)	Same
Reaction temperature	37°C	Same

Differences		
Item	Device	Predicate
Instrumentation	ImmunoCAP 100 and 250 (fully automated)	Gamma Counter
Assay type	ELISA	Radioassay
Internal Controls	Positive and Negative controls provided within the EliA ANA	Positive and Negative controls

Differences		
Item	Device	Predicate
	control kit, sold separately.	included in the kit
Calibration curve	Option to store curve for up to 28 days and run curve controls (provided in kit) in each assay for calibration	6-point calibration curve to be run with every test
Signal	Fluorescence	Counts per minute
Detection antibody (antibody)	Anti-human IgG β -galactosidase (mouse monoclonal antibodies)	Room temperature, 18-25°C
Concept	Modular reagents concept (test-method specific and general reagents)	None

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

None referenced.

L. Test Principle:

The EliA dsDNA wells are coated with double-stranded plasmid DNA. If present in the patient's specimen, antibodies to gliadin will bind to their specific antigen in the wells. After washing away non-bound 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.

The EliA IgG calibration is a total IgG calibration. It is based on a set of six WHO-standardized IgG calibrators derived from human serum. The calibrators are required to perform an initial calibration curve, which can be stored in the ImmunoCAP instrument and may be used up to 28 days. Each assay outside of a calibration run includes curve controls that have to fall within defined ranges to verify that the stored calibration curve is still valid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. ***Precision/Reproducibility:***

For the ImmunoCAP 100, three samples were measured on three instruments in 36 runs with a calibration curve in each run. For the ImmunoCAP 250, three samples were measured on three instruments in 3 replicates in 21 runs with a calibration curve in each run.

ImmunoCAP 100

Sample	Mean (U/mL)	Intra-run (CV %)	Inter-run (CV %)
1	18.6	3.6	3.7
2	56.6	4.1	3.3
3	102	4.0	2.8

ImmunoCAP 250

Sample	Mean (U/mL)	Intra-run (CV %)	Inter-run (CV %)
1	9.8	5.3	4.6
2	15.1	5.0	3.2
3	81.5	2.8	4.6
4	209.4	5.3	4.5

Additional studies were done on three ImmunoCAP 100 instruments for equivocal samples around 15 IU/mL and a high positive sample. The samples were tested in 3 replicates in 18 runs (total 54 replicates) over 6 days. The studies yielded the following:

Sample	Mean value (IU/mL)	Coefficients of variation (%)	
		Intra-Run	Inter-Run
1	8.9	4.9	4.2
2	9.3	4.3	5.0
3	9.6	5.8	5.0
4	11.3	6.1	7.2
5	13.1	3.3	6.9
6	273.3	5.4	7.8

b. Linearity/assay reportable range:

Linearity was not claimed for this device.

High dose hook effect:

For EliA dsDNA, the possibility of antigen excess occurring when using the device was evaluated with serum sample above the calibrator 600 ug/mL. The device was able to discriminate the high positive sample diluted 1:10 (~6,000 ug/L) from the highest calibrator point showing no hook effect.

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

The IgG calibrators are traceable to the International Reference Preparation (IRP) 67/86 of Human 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 (6 levels). The instrument measures specific IgG concentrations in µg/L. To obtain a test specific result, µg/L of IgG must be converted to EliA U/mL using a conversion factor given by the lot-specific code of the EliA dsDNA well.

EliA ANA Control is prepared from selected pooled human sera containing IgG antibodies to dsDNA, RNP, Sm, Ro, La, Scl-70, CENP and Jo-1. The controls are prediluted and ready for use. The acceptance ranges for the current control lot are stated on the Control Certificate included in the respective EliA ANA Control kit. The mean values for every lot have been determined with 4 consecutive control assays, each in 6 replicates. Ranges are

calculated as respective mean \pm 3SD for the expected long term variation.

Open-vial stability is not tested as the EliA ANA control is packaged in single vials. Closed-vial stability studies were performed to investigate the accelerated stability claim of 24 months. According to internal specifications 4 weeks at +30°C correspond to one year storage at +4°C and 8 weeks at +30°C correspond to two years storage at +4°C. For approved stability, the quotas (stressed/reference) for positive signal should be within 0.80 to 1.20. Specifications were met.

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 (4.0 µg/L) in the Calibrator Wells. The results in Response Units (RU) were compared with the result of the sample diluent on EliA dsDNA Wells. The discrimination ability (D) of the assay should be >2.0. All samples were measured in triplicate.

Sample ID	Results on Calibrator Wells	
	Mean Response Units (RU)	SD
Calibrator 4.0 (1:2)	258	4.8
Calibrator 4.0 (1:4)	163	4.8
Calibrator 4.0 (1:8)	119	3.3

Sample ID	Results on dsDNA Wells	
	Mean RU	SD
Sample Diluent	1	0.4

The 1/8 diluted calibrator 4.0 (0.5 µg/L) still can be discriminated from background given by the signal of the diluent on dsDNA wells. The lower limit of detection was set at 0.5µg /L.

e. Analytical specificity:

Interfering substance

Two diluted positive serum samples were spiked with bilirubin C, bilirubin F, hemoglobin, chyle and rheumatoid factor. The same samples were also spiked with specific blanks. 990 µL diluted serum was spiked with 10 µL substance or blank. For rheumatoid factor, 900 µL serum were spiked with 100 µL interference substance. The samples were tested in 3 replicates. A calibration curve was run in each assay. The runs were repeated twice. The following sample concentrations of additives were reached.

Additives	Concentration in raw sample	Final concentration in diluted sample (1:100)	Normal Values
Bilirubin F	21.1 mg/dL	2.11	<1.0
Bilirubin C	20.6 mg/dL	2.06	<1.0

Additives	Concentration in raw sample	Final concentration in diluted sample (1:100)	Normal Values
Chyle	157,000 Units/dL	15,700	No data
Hemoglobin	519 mg/dL	51.9	<2.0
Rheumatoid Factor IgM	500 IU/mL	50	<40.0

The specification was set such that the ratio of the result of the sample spiked with the interfering substance and the sample spiked with a buffer blank should be between 0.8 and 1.2. The tables below show the results of the study.

Equivocal Sample

Additive	Blank/spiked sample	Run 1			Run 2		
		Conc. U/mL	CV %	Ratio	Conc. (U/mL)	CV %	Ratio
Bilirubin F	Blank	9.2	9.3	0.97	8.0	2.9	0.93
	Sample	8.9	4.2		7.5	6.0	
Bilirubin C	Blank	8.2	7.5	0.98	7.7	6.7	0.96
	Sample	8.0	4.9		7.4	5.5	
Hemoglobin	Blank	8.4	2.3	1.01	8.0	0.7	0.95
	Sample	8.5	7.3		7.5	3.4	
Chyle	Blank	9.1	9.6	0.97	7.5	5.5	1.00
	Sample	8.9	5.6		7.5	4.7	
RF	Blank	9.4	8.4	0.96	7.8	1.5	1.00
	Sample	9.0	2.1		7.8	5.8	

Positive sample

Additive	Blank/spiked sample	Run 1			Run 2		
		Conc. U/mL	CV %	Ratio	Conc. (U/mL)	CV %	Ratio
Bilirubin F	Blank	83.8	2.3	0.96	84.8	4.2	0.97
	Sample	80.3	7.1		82.4	4.4	
Bilirubin C	Blank	82.3	4.3	1.01	85.7	6.5	1.02
	Sample	83.6	1.5		87.7	1.8	
Hemoglobin	Blank	88.2	0.5	0.97	90.4	0.7	0.98
	Sample	85.7	1.2		88.4	3.3	
Chyle	Blank	85.0	1.3	0.99	86.6	5.5	0.89
	Sample	84.5	2.2		77.1	1.3	
RF	Blank	92.7	3.4	0.96	87.8	1.7	0.95
	Sample	88.8	3.5		83.6	6.4	

Two additional serum samples showing values around the cut-off were spiked

with bilirubin C, bilirubin F, hemoglobin, chyle and rheumatoid factor. The same samples were also spiked with substance specific blanks. The samples were tested in three replicates and repeated twice. The same specification as above was set. Results are shown below:

Equivocal Sample 1		Run 1			Run 2		
Additive	blank/spiked sample	Conc. [IU/mL]	CV %	Ratio	Conc. [IU/mL]	CV %	Ratio
Bilirubin F	blank	15.5	9.1	0.91	14.7	7.1	0.95
	sample	14.1	8.2		14.0	6.9	
Bilirubin C	blank	13.9	13.4	1.05	13.4	8.1	1.02
	sample	14.6	1.9		13.7	6.6	
Hemoglobin	blank	15.4	6.6	0.92	15.5	7.0	0.87
	sample	14.1	3.6		13.5	7.2	
Chyle	blank	14.1	8.7	1.01	12.5	9.8	1.04
	sample	14.3	7.5		13.0	5.2	
Rheumatoid factor	blank	15.8	5.5	1.17	15.8	2.9	1.12
	sample	18.5	10.1		17.7	1.7	

Equivocal Sample 2		Run 1			Run 2		
Additive	blank/spiked sample	Conc. [IU/mL]	CV %	Ratio	Conc. [IU/mL]	CV %	Ratio
Bilirubin F	blank	11.5	10.6	1.03	10.9	4.7	1.02
	sample	11.9	6.4		11.1	3.4	
Bilirubin C	blank	11.1	7.2	0.95	10.9	6.8	0.96
	sample	10.6	2.3		10.4	3.2	
Hemoglobin	blank	10.0	8.7	1.13	11.3	4.4	0.97
	sample	11.2	3.6		11.0	2.3	
Chyle	blank	11.5	10.8	0.88	11.3	2.1	1.03
	sample	10.1	10.8		11.6	4.5	
Rheumatoid factor	blank	10.0	1.6	1.04	10.4	5.9	0.92
	sample	10.4	4.3		9.6	0.8	

The interfering substances listed did not appear to adversely affect the results of the new devices.

Cross-reactivity

Panels of international reference sera from (Centers of Disease Control and Association of Medical Laboratory Immunologists) were selected to show analytical specificity of the device. The samples were analyzed in duplicate using one batch of EliA dsDNA wells and one batch of system reagents. A calibration curve was run in duplicate. The results are shown on the tables below:

CDC ANA Human Reference Panel

Sample	IFA Pattern	Target	Diagnosis	IU/mL
CDC1	Homogeneous/rim	dsDNA & weak Sm	Not available	217.8
CDC2	Speckled	SS-B/La	Not available	0.5
CDC3	Speckled	UI-RNP, SS-A/Ro, SS-B/La	Not available	1.1
CDC4	n.a	UI-RNP	Not available	1.2
CDC5	n.a	SM,histone	Not available	15.1
CDC6	Nucleolar	n.a	Not available	0.9
CDC7	n.a	SS-A/Ro	Not available	5.1
CDC8	centromere	CENP	Not available	2.3
CDC9	n.a	Scl-70	Not available	3.4
CDC10	n.a	Jo-1	Not available	1.1

CDC5 is reported to be high positive for Sm Abs. and also contains histone Abs (Tan et.al.1999) but was found positive for dsDNA by EliAdsDNA. Sm is well known as marker for SLE, hence it is very likely that CDC5 belongs to a SLE patient. DsDNA is also known to be SLE marker and is very often associated with a high Sm titer.

AML I Reference Panel 2001

Sample	Target	Diagnosis	IU/mL
AML I A	CENP	CREST	2.0
AML I B	Scl-70	Scleroderma	3.1
AML I D	UI-RNP	MCTD	1.4
AML I E	SS-A/Ro	Sjogren's syndrome, SLE	1.6
AML I F	Jo-1,SS-A/Ro	Polymyositis	1.2
AML I G	SS-B/La,SS-A/Ro	Sjogren's syndrome	2.0
AML I I	Sm, UI-RNP,dsDNA	SLE	26.6
AML I J	dsDNA, Ro	SLE	8.5
AML I K	Negative	healthy	0.0
AML I L	Negative	healthy	0.1

AML I J is derived from a SLE patient and described as exclusively dsDNA positive. The negative result with the device may be due to the stringent washing procedure of EliA combined with a low affinity of the dsDNA antibodies in the AML I J serum.

No other cross reactivity to other autoantibodies was detected.

f. Assay cut-off:

See expected values.

2. Comparison studies:

a. Method comparison with predicate device:

Clinical samples:

One hundred six patient samples covering the measuring range were tested with the EliA dsDNA and the predicate devices, DPC RIA. These samples included 60 SLE and 46 non-SLE patients. Non-SLE samples included patients with rheumatoid arthritis (20), progressive systemic sclerosis (5),

CREST- limited systemic sclerosis (1), Hepatitis C virus (1), mixed connective tissue disease- MCTD (3), healthy controls (16). Equivocal results (nine SLE patients) were excluded from calculation. Results showed the following:

n = 97		DPC RIA		
		positive	negative	Total
EliA dsDNA	positive	48	0	48
	negative	5	44	49
	Total	53	44	97

Total agreement = 94.8% (92/97) (95%CI 88.4-98.3)

Positive % agreement = 90.6% (48/53) (95%CI 79.3-96.9)

Negative% agreement = 100.0% (44/44) (95%CI 92-100)

b. Matrix comparison:

Forty six sets of samples from different donors were tested in double determinations. Sample demographics were not provided. Each set contained serum, EDTA, heparin and citrate plasma samples. For positive and equivocal serum samples quotas between serum and each type of plasma were calculated. Mean quota of plasma to serum concentration should be 0.8-1.2 for positive sera. Negative samples should not switch to positive in all serum and plasma samples. Linear regression comparing the quotas between serum and each type of plasma for the positive samples was performed and showed:

	n	Slope	Intercept	Correlation Coefficient
Serum vs. Plasma Citrate	46	1.040 (95%CI: 1.00, 0.086)	-0.157 (95%CI: -0.250, 0.043)	0.997
Serum vs. Plasma Heparin	46	1.067 (95%CI: 1.025, 1.161)	0.264 (95%CI: 0.133, 0.393)	0.997
Serum vs. Plasma EDTA	46	1.015 (95%CI: 0.981-1.051)	0.106 (95%CI: 0.007, 0.199)	0.995

The specifications for this study are fulfilled for serum, heparin, EDTA and citrate plasma samples. This information is specified on the Specimen Collection section of the Package Insert.

c. Instrument Platform comparison:

The purpose of this study was to demonstrate that the performance of EliA dsDNA is equivalent on the ImmunoCAP 100 and the ImmunoCAP 250. For this comparison study, a total of 59 samples distributed over the measuring range were assayed: 4 negative samples, 32 positive samples and 23 high positive samples. All samples were run on three ImmunoCAP 100 instruments and two ImmunoCAP 250 instruments in two runs and in single replicates. The specification for correlation is that the systems are considered equal if the true average difference is less than $\pm 5\%$ with no linear trends and

no sample related differences (with the same lot) of reagents. The study yielded the following:

	n	Slope	Intercept	Correlation Coefficient
IC100 vs. IC 250	59	0.979 (95%CI: 0.929, 1.022)	0.532 (95%CI: -1.875, 3.950)	0.986

3. Clinical studies:

a. *Clinical Sensitivity and Clinical Specificity:*

None provided.

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

Not applicable.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

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 sex and age. Results were tabulated on the table below:

	IU/mL
Median	1.4
Mean	2.4
Mean +2SD	10.7
Mean +3SD	14.9
95 th Percentile	6.3
99 th Percentile	17.3

The results appeared to be equally distributed and not dependent on age or gender. The 95th percentile lies below the lower limit of the equivocal range of 10-15 IU/mL.

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.