

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

A. 510(k) Number:

k132631

B. Purpose for Submission:

New device

C. Measurand:

IgG antibodies specific for Sm protein

D. Type of Test:

Fluoroenzymeimmunoassay, Semi-quantitative

E. Applicant:

Phadia US Inc.

F. Proprietary and Established Names:

EliA™ SmD^P Immunoassay

G. Regulatory Information:

1. Regulation section:
21 CFR §866.5100 Antinuclear Antibody Immunological Test System
2. Classification:
Class II (Assay)
3. Product code:
LKP, Anti-Sm Antibody, Antigen and Control
4. Panel:
Immunology (82) (Assays)

H. Intended Use:

1. Intended use(s):

EliA SmD^P is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Sm in human serum and plasma (EDTA, citrate) to aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA SmD^P uses the EliA IgG method on the instrument Phadia 100.

EliA SmD^P is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Sm in human serum and plasma (EDTA, citrate) to aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA SmD^P uses the EliA IgG method on the instrument Phadia 250.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Phadia 100 and Phadia 250 instruments

I. Device Description:

EliATM uses a modular reagent system. The test specific, method specific and general reagents are packaged and purchased as separate units. The reagents on Phadia® 100/Phadia® 250 are identical; they are only filled in different containers.

EliASmD^P Test-Specific Reagents consist of:

- 1) EliATM SmD^P wells coated with synthetic SmD3 peptide
- 2) EliATM SmD^P Positive Control, containing human monoclonal antibodies specific to SmD3 peptide
- 3) EliATM IgG/IgM/IgA Negative Control containing normal human serum from healthy donors.

The control materials were cleared k072393 and k131821.

Also required for the test are EliATM Method-Specific Reagents:

EliATM IgG Calibrators (human IgG in PBS (0, 4, 10, 20, 100, 600 µg/L), EliATM IgG Curve Control (human IgG (5µg/L) in PBS), EliATM Sample Diluent (PBS containing BSA, detergent and 0.095% sodium azide), EliATM IgG Conjugate (β-galactosidase labeled mouse monoclonal anti- human IgG).

J. Substantial Equivalence Information:

1. Predicate device name(s):

Varelisa Sm Antibodies

2. Predicate 510(k) number(s):

k042629

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use / Indications for Use	EliA SmD ^P is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Sm in human serum and plasma (EDTA, citrate) to aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA SmD ^P uses the EliA IgG method on the instruments Phadia 100 and Phadia 250.	The Varelisa Sm Antibodies EIA kit is designed for the semiquantitative and qualitative determination of SmD antibodies in serum or plasma to aid in the diagnosis of systemic lupus erythematosus (SLE).
Assay Type	ELISA	Same
Type of Test	Semi-quantitative	Same
Antigen	Synthetic SmD3 peptide	Same
Solid Phase	Microwells	Same

Differences		
Item	Device	Predicate
Instrumentation	Phadia 100 and 250 are fully automated immunoassay analyzers	ELISA Reader needed
Controls	Positive and Negative Controls sold separately	Positive and Negative Control Sera provided with assay
Reaction temperature	37°C (controlled)	Room temperature, 18-25°C
Incubation times	Diluted patient samples: 30 min. Conjugate: 28 min.	Calibrators, Controls, diluted patient samples: 30

Differences		
Item	Device	Predicate
	Development Solution: 39 min.	min. Conjugate: 30 min. Substrate: 10 min.
Detection antibody (conjugate)	mouse monoclonal anti-human IgG β -Galactosidase	rabbit polyclonal anti-human IgG horse-radish peroxidase
Signal	Fluorescence	Optical density
Calibration	Option to store total IgG calibration curve for up to 28 days and run curve controls in each assay for calibration	Analyte-specific IgG calibration curve in each test
Calibrators	Set of six vials of human IgG at concentrations of 0, 4, 10, 20, 100, and 600 μ g/L	Set of six vials of Sm antibody calibrators at concentrations of 0, 3, 7, 16, 40, and 100 U/mL
Sample Dilution	1:50	1:101
Reportable Range	0.8 U/mL – 480 U/mL	0.5 U/mL - 100 U/mL
Cut-off/ Results Interpretation	<7 U/mL negative 7-10 U/mL equivocal >10 U/mL positive	<10 U/mL negative 10-15 U/mL equivocal >15 U/mL positive

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

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The EliA SmD^P Wells are coated with a synthetic SmD3 peptide. If present in the patient's specimen, antibodies to the SmD3 peptide bind to their specific antigen. 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 higher the amount of antibody bound and detected in the sample tested. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

The total IgG calibration is based on a set of six WHO-standardized IgG Calibrators derived from human serum. They are used to establish an initial calibration curve, which may be used for up to 28 days on additional assays and can be stored by the instrument. Each additional assay includes calibrator (curve) controls that have to recover in defined ranges to ensure that the stored calibration curve is still valid. Limits for the response of the Curve Controls are defined in the Phadia 100/250 Operator and Panel Software.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of the EliA SmD^P assay on the Phadia 100 was assessed in a study with 8 samples. Each sample was tested over 7 runs, 4 replicates/run on 3 Phadia 100 instruments over 7 days for a total of 84 replicate determinations per sample). A new calibration curve was performed on each run. The results are presented in the table below.

EliA SmD^P on Phadia 100			
Mean (U/mL)	Intra run (CV%)	Inter run (CV%)	Total
8.5	4.8	2.5	5.4
17.8	6.0	3.5	6.0
364.7	6.4	4.5	7.8
4.0	11.2	14.3	18.2
11.5	3.8	4.4	5.8
39.9	3.2	4.2	5.3
82.1	2.7	3.9	4.7
293.5	5.0	6.1	7.8

The precision of the assay on the Phadia 250 was assessed as described above for the Phadia 100 instrument.

EliA SmD^P on Phadia 250			
Mean (U/mL)	Intra run (CV%)	Inter run (CV%)	Total
7.7	6.5	1.6	6.7
17.5	5.3	3.4	6.3
377.7	4.0	2.6	4.7

For all samples, the inter-run and intra-run coefficients of variation of EliA SmD^P were within the sponsor's acceptance criteria (<10% and <8% respectively).

To determine the lot-to-lot reproducibility of the assay on the Phadia 250, 3 samples were tested using 3 lots, over 7 runs (1 run/day), 4 replicates/run on 3 Phadia 250 instruments for a total of 252 repetitions. A calibration curve was included in each run. The results are presented in the table below.

EliA SmD^P Lot-to-Lot Reproducibility	
Mean (U/mL)	Reproducibility (CV%)
7.7	3.8
17.5	9.5
377.7	1.1

b. Linearity/assay reportable range:

Six patient serum samples were diluted to produce 7 levels of reactive specimens that were analyzed to assess linearity.

Linearity of EliATM SmD^P on Phadia 100					
Sample	Dilution range (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	%CV Range
1	9.2- 78.3	0.99 (0.96 to 1.02)	0.08 (-2.03 to 0.62)	1.00	0.4 - 3.0
2	3.7 - 10.2	1.24 (1.20 to 1.28)	-1.42 (-1.70 to 1.14)	1.00	2.21 - 4.5
3	9.7 - 207.5	1.067 (1.02 to 1.11)	-0.67 (-4.91 to 3.57)	1.00	1.5 - 5.4
4	5.2 - 252.7	1.04 (1.01 to 1.08)	0.92 (-2.61 to 4.45)	1.00	0.6 - 6.1
5	6.5 - 398.3	1.02 (0.99 to 1.05)	2.41 (-2.17 to 7.00)	1.000	0.6 - 7.4
6	9.2 - 78.3	0.99 (0.96 to 1.02)	-0.7051 (-2.03 to 0.62)	1.000	1.4 - 3.0

Linearity of EliATM SmD^P on Phadia 250					
Sample	Dilution range (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	%CV Range
1	5.5 - 74.3	1.04 (1.03 to 1.05)	-1.03 (1.36 to 0.70)	1.00	0.3 - 4.5
2	3.8 - 281.3	1.09 (1.03 to 1.14)	1.91 (-3.75 to 7.57)	1.00	0.9 - 5.3
3	6.6 - 210.7	1.09 (1.08 to 1.10)	-2.54 (-3.88 to -1.20)	1.00	0.9 - 7.2
4	8.8 - 227.4	1.06 (1.04 to 1.09)	1.192 (-0.95 to 3.34)	1.00	0.5 - 6.5
5	6.1 - 419.8	1.12 (1.11 to 1.13)	-0.36 (-2.20 to 1.47)	1.00	1.3 - 4.5
6	5.5 - 74.3	1.04 (1.03 to 1.05)	-1.03 (-1.36 to -0.70)	1.00	0.3 - 4.5

The limits of the measuring range were set to 0.8 - >480 U/mL. The upper limit of the reportable range can vary between 480 and 720 U/ml, due to a lot-specific conversion

from µg/L to EliA U/mL. Results above the upper limit are reported as “above”.

High dose hook effect: A hook effect was not observed when analyzing a high positive sample that had a concentration up to 8 times above the upper limit of the measuring range.

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

i) *Traceability:*

Calibrators: There is no international reference standard for IgG antibodies that specifically recognize Sm or the SmD3 peptide. The instrument measures specific IgG concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA SmD^b Well, the results are automatically converted to EliA Units/mL.

EliA IgG Calibrators and Curve Controls are traceable (via an unbroken chain of calibrations) to the International Reference Preparation (IRP) 67/86 of 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.

The calibrators are required to perform an initial calibration curve, which can be stored in the Phadia instrument and may be used for up to 28 days on this and additional IgG assays. Each additional 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.

Controls: EliA ANA Positive Control is prepared from selected pooled human sera and contains IgG antibodies to dsDNA, RNP, Sm, Ro, La, Scl-70, CENP and Jo-1. This control was cleared under k072393. The EliA™ IgG/IgM/IgA Negative Control was prepared from normal human serum. This control was cleared under k131821. The target ranges for the EliA™ Controls for the two platforms are summarized below:

Instrument	EliA™ Positive SmD^b Control
Phadia 100	31.7 – 73. U/mL
Phadia 250	31.1 – 72.5 U/mL
Instrument	EliA™ IgG/IgM/IgA Negative Control
Phadia 100	≤ 4 U/mL
Phadia 250	≤ 4 U/mL

ii) *Kit Stability:*

Closed and open stability - An accelerated stability study initially determined the shelf-life of the EliA SmD^P Well was 24 months. A real-time stability study supported a 24 months stability claim. All studies were performed on three batches of EliA SmD^P Well. Other required components (previously reviewed) of the assay method have a shelf life of 18 to 24 months. The sponsor notes that it is important to store the wells in dry conditions at 2-8°C.

On-board stability - The on-board stability of the EliA SmD^P Wells packed in carriers were tested for 2, 4 and 6 weeks at 10 °C and 80% humidity in duplicates in one run using 3 positive and 2 negative samples only on the Phadia 250 instrument since for Phadia 100 instrument the reagents are stored outside the instrument and are only loaded as needed for an assay. The on-board stability for the EliATM SmD^P Wells was determined to be 28 days at 2-8°C.

iii) *Sample Storage*

The sponsor recommends following the guidelines in CLSI H18-A3 for sample storage. Separated serum/plasma should remain at room temperature for no longer than eight hours. If assays will not be completed within eight hours, serum/plasma should be refrigerated (2 to 8°C). If assays are not completed within 48 hours, or the separated serum/plasma will be stored beyond 48 hours, serum/plasma should be frozen at or below -20°C. Freezing and thawing should be avoided.

d. *Detection limit:*

The Limit of Blank (LOB), Limit of Detection (LOD) and Limit of Quantitation were determined based on CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, EP17-A”. On each system (Phadia 100 and Phadia 250), six samples with undetectable levels of analyte were spiked with EliA Sample Diluent and were measured in 72 replicates. The sample with the lowest value was chosen to be the blank sample while the other 5 samples were deemed ‘low positives’ for this experiment. For Phadia 100 and Phadia 250, each sample was run in a total of six runs, three runs in two instruments.

The values determined are presented here:

EliATM SmD^P	LoB	LoD	LoQ
Phadia 100	0.39 U/mL	0.69 U/mL	0.69 U/mL
Phadia 250	0.45 U/mL	0.73 U/mL	0.73 U/mL

e. *Analytical specificity:*

Comparison to Reference Sera: Twelve reference sera were obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA. These sera have known reactivity for a number of autoantibodies that include DNA, SS-B/La, U1-RNP, SS-A/Ro, PCNA, Pol III, PM/Scl, CENP, Scl-70, Jo-1, PM/Scl, rRNP/Rib P. In addition,

two of the reference sera were known to be positive for Sm. Among the 12 sera, only the two sera that were known to be positive for Sm were found to be reactive in the EliA SmDP assay.

Endogenous Interference: Interferences were assessed by testing three positive serum samples: two samples around the cut-off (11 and 12 U/mL), and a high positive (>110 U/mL). Each sample was spiked with the interfering substances or substance-specific blanks and analyzed using one lot of EliA SmD^P Well and one lot of system reagents in two runs, each in three replicates (n=6). The data demonstrated that SmD^P was not adversely affected by high levels of the following substances tested up to the concentrations listed in the table below:

Potential Interfering Compound	Concentration
Bilirubin F	19.2 mg/dL
Bilirubin C	20.1 mg/dL
Hemoglobin	496 mg/dL
Lipemic factor (ClinOleic)	1 g/dL (1%)
Rheumatoid factor	500 IU/mL

f. Assay cut-off:

Based on the results of the expected values/reference range study described below in Section M.5, the 99th percentile lies below the upper limit of the equivocal range for the EliA SmD^P assay. The cut-off between equivocal and positive was set to 10 U/mL. The assay cutoffs were set as follows:

Decision Point	Interpretation
<7 U/mL	Negative
7-10 U/mL	Equivocal
>10 U/mL	Positive

Where specimens yield equivocal results, the sponsor recommends retesting the patient again after 6-8 weeks.

2. Comparison studies:

a. Method comparison with predicate device:

One hundred twenty samples from individuals diagnosed with SLE and 250 specimens from individuals with other autoimmune and infectious conditions were tested (see 3.a. below for a detailed list). In addition, 20 specimens with known anti-Sm reactivity, but without clinical diagnosis, were added to the analysis.

Of the 370 specimens tested, 16 were excluded as being outside of the measuring range for one or both methods. For analysis of agreement with the predicate device, 354 sera were measured on the Phadia 250 instrument. All samples were within the reportable range for both assays.

		Varelisa Sm (Sm Units)			
		Positive: >15	Indeterminate: 10-15	Negative: <10	Total
EliA™ SmD ^P (U/mL)	Positive: >10	39	4	2	45
	Equivocal : 7-10	2	0	3	5
	Negative: <7	1	10	293	304
	Total	42	14	298	354

Agreements were calculated by grouping each assays' equivocal results with its test negative results, and then agreement was calculated again by grouping each assays' equivocal results with the test positive results:

Equivocal results considered as negative		Varelisa Sm (Sm Units)		
		Positive: >15	Negative: <15	Total
EliA SmD ^P (U/mL)	Positive: >10	39	6	45
	Negative: <10	3	306	309
	Total	42	312	354

Positive percent agreement: 92.9% (39/42) 95% CI: 80.5 - 98.5%
 Negative percent agreement: 98.1% (306/312) 95% CI: 95.9 - 99.3%
 Total percent agreement: 97.5% (345/354) 95% CI : 95.2 - 98.8%

Equivocal results considered as positive		Varelisa Sm (Sm Units)		
		Positive: >10	Negative: <10	Total
EliA SmD ^P (U/mL)	Positive: >7	45	5	50
	Negative: <7	11	293	304
	Total	56	298	354

Positive percent agreement: 80.4% (45/56) 95% CI: 67.6 - 89.8%
 Negative percent agreement: 98.3% (293/298) 95% CI: 96.1 - 99.5%
 Total percent agreement: 95.5% (338/354) 95% CI : 92.8 - 97.4%

b. Matrix comparison:

Twenty-five negative subjects and twenty-five positive subjects provided sera and plasma specimens (EDTA and citrate) and these were tested for SmD^P on the Phadia 250. The sponsor's acceptance criteria were that each plasma sample should yield results within 20% of the serum specimen and that all negative specimens should remain negative. A Passing & Bablok analysis was performed and the results are depicted in the following table:

	Range tested (U/mL)	Slope (95% CI)	Intercept (95% CI)	R²
Serum vs. Citrate plasma	0.5 – 433.9	0.99 (0.98 to 1.01)	-0.09 (-0.82 to 0.15)	1.00
Serum vs. EDTA plasma	0.5 – 413.1	1.00 (0.97 to 1.01)	0.19 (-0.39 to 0.70)	1.00

c. *Instrument comparison:*

In order to determine if the performance of EliA SmD^P is equivalent on the instruments Phadia 100 and Phadia 250, 36 samples distributed over the measuring range were tested: 25 positive, 7 equivocal and 4 negative samples. All samples were run on 3 different Phadia 100 and 3 different Phadia 250 instruments. Each sample was tested once on each instrument. The results from Phadia 100 Instrument 1 were compared to the results from Phadia 250 Instrument 1, and so on for the other two instrument pairs. The results of a Weighted Deming regression analyses performed on each pair are shown below.

EliA™ SmD^P Weighted Deming Regression: Phadia 100 vs Phadia 250		
Instrument Pair	Slope (95% CI)	Y-Intercept (95% CI)
1	0.95 (0.92 to 0.97)	-0.78 (-1.13 to -0.44)
2	0.97 (0.92 to 1.01)	-0.64 (-1.33 to 0.05)
3	0.98 (0.94 to 1.03)	-0.47 (-1.04 to 0.10)

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

One hundred samples from individuals diagnosed with SLE and 250 specimens from individuals with other autoimmune and infectious conditions were tested on the Phadia 250 instrument.

Equivocal results considered as positive		SLE Diagnosis		
		Positive:	Negative:	Total
EliA SmD^P (U/mL)	Positive: > 10	75	4	79
	Equivocal: 7-10	2	2	4
	Negative: < 7	23	244	267
	Total	100	250	350

Clinical sensitivity and specificity were calculated by grouping the assay's equivocal results with its test negative results, and then sensitivity and specificity was calculated again by grouping the assay's equivocal results with the test positive results:

Equivocal results considered as negative		SLE Diagnosis		
		Positive:	Negative:	Total
EliA SmD ^P (U/mL)	Positive: >10	23	4	27
	Negative: <10	77	246	323
	Total	100	250	350

Sensitivity: 23.0% (23/100) 95% CI: 15.2 – 32.5%
 Specificity: 98.4% (246/250) 95% CI: 96.0 - 99.6%

Equivocal results considered as positive		SLE Diagnosis		
		Positive:	Negative:	Total
EliA SmD ^P (U/mL)	Positive: >7	25	6	31
	Negative: <7	75	244	319
	Total	100	250	350

Sensitivity: 25.0% (25/100) 95% CI: 16.9 - 34.7%
 Specificity: 97.6% (244/250) 95% CI: 94.8 – 99.1%

Performance on Non-SLE Specimens:

One hundred fifty specimens from individuals having other conditions were tested for SmD^P reactivity, including dermatomyositis, polymyositis, scleroderma, mixed connective tissue disease, rheumatoid arthritis, Hashimoto thyroiditis, Graves' Disease and Sjögren's Syndrome. In this study, no more than 5% of the specimens that were tested were found to be positive for SmD^P at a level > 10 U/mL.

Non-SLE Patient Sub-Group	Performance on Non-SLE Specimens		
	N	No (%) Positive* on EliA SmD ^P	No (%) Positive* on predicate
Dermatomyositis	4	0 (0%)	0 (0%)
Polymyositis	6	0 (0%)	0 (0%)
Scleroderma	30	1 (3%)	1 (3%)
MCTD	20	1 (5%)	0 (0%)
RA	50	1 (2%)	2 (4%)
Hashimoto	10	0 (0%)	0 (0%)
Graves' Disease	10	0 (0%)	0 (0%)
Sjögren's Syndrome	20	0 (0%)	0 (0%)
Bacterial infections	30	0 (0%)	0 (0%)
Viral infections	30	0 (0%)	1 (3%)

* Equivocal samples (2) were considered negative for this analysis

b. Other clinical supportive data:

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Four hundred samples from healthy Caucasian individuals were measured on the Phadia 250 instrument. The results are presented in the following table:

	U/mL
Mean	1.4
Median	2.8
Range	0.1 – 9.1
95th percentile	3.0
99th percentile	6.7

The results were equally distributed and not dependent on age or gender. No samples were positive (>10 U/mL) in this study, but 3 (0.75%) of the samples fell in the equivocal range. Expected values may vary depending on the population tested. A subset (n=70) of the samples tested on the Phadia 100 gave similar results.

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.