

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

**A. 510(k) Number:**

k140493

**B. Purpose for Submission:**

New device

**C. Measurand:**

IgG antibodies specific for Scl-70 protein

**D. Type of Test:**

Fluoroenzyme immunoassay, Semi-quantitative

**E. Applicant:**

Phadia US Inc.

**F. Proprietary and Established Names:**

EliA™ Scl-70<sup>S</sup> Immunoassay

**G. Regulatory Information:**

1. Regulation section:

21 CFR §866.5100, Antinuclear Antibody Immunological Test System

2. Classification:

Class II (Assays)

3. Product code:

LJM, Antinuclear Antibody (Enzyme-Labeled), Antigen, Control

4. Panel:

Immunology (82) (Assays)

**H. Intended Use:**

1. Intended use(s):

EliA™ Scl-70<sup>S</sup> is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Scl-70 in human serum and plasma (heparin, EDTA) as an aid in the clinical diagnosis of sclerodema (diffuse form) in conjunction with other laboratory and clinical findings. EliA™ Scl-70<sup>S</sup> uses the EliA™ IgG method on the instrument Phadia 100.

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2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use on the instruments Phadia® 100 and Phadia® 250 (k061165).

**I. Device Description:**

EliA™ 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 and Phadia® 250 are identical; they are only filled in different containers.

EliA™ Scl-70<sup>S</sup> Test-Specific Reagents consist of:

- 1) EliA™ Scl-70<sup>S</sup> wells coated with human recombinant Scl-70 protein. The EliA™ wells are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant.
- 2) EliA™ ANA Positive Control containing IgG antibodies to dsDNA, RNP, Sm, Ro, La, Scl-70, CENP and Jo-1 in human serum.
- 3) EliA™ IgG/IgM/IgA Negative Control containing normal human serum from healthy donors.

Also required for the test are EliA™ Method-Specific Reagents:

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

**J. Substantial Equivalence Information:**

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

QuantaLite™ Scl-70 ELISA, (k924898)

2. Comparison with predicate:

Item	Similarities	
	New Device EliA™ Scl-70 <sup>S</sup>	Predicate QuantaLite™ Scl-70 ELISA
Intended Use/Indications for Use	Measurement of IgG antibodies directed to Scl-70 to aid in the clinical diagnosis of scleroderma in conjunction with other laboratory and clinical findings	Same
Type of Test	Semi-quantitative	Same
Solid Phase	Polystyrene microwells	Same

<b>Differences</b>		
<b>Item</b>	<b>New Device EliA™ Scl-70<sup>S</sup></b>	<b>Predicate QuantaLite™ Scl-70 ELISA</b>
Form of scleroderma	Diffuse form	Diffuse and limited forms
Assay Type	Automated fluoroenzymatic immunoassay	Manual ELISA
Coating Antigens	Human recombinant Scl-70 protein	Purified Scl-70 antigen
Sample Matrix	Serum and plasma (heparin and EDTA)	Serum
Sample Dilution	1:100 (manual or instrument dilution)	1:101 (manual dilution only)
Reaction Temperature	37°C (controlled)	Room temperature (20-25°C)
Incubation times	Diluted patient samples: 30 min. Conjugate: 28 min. Development Solution: 39 min.	High positive, low positive and negative controls, diluted patient samples: 30 min. Conjugate: 30 min. Substrate: 30 min (in dark).
Detection Antibody (Conjugate)	Mouse monoclonal anti-human IgG β-Galactosidase	Goat anti-human IgG horseradish peroxidase
Substrate/Chromogen	4-Methylumbelliferyl-βD-Galactoside (MUG)	Tetramethylbenzidine (TMB)
Stop Solution	Sodium Carbonate (4%)	Sulfuric Acid (0.344 M)
Signal	Fluorescence	Optical density
Instrumentation	Phadia 100 and 250 are fully automated immunoassay analyzers	Microwell plate reader (450 nm)
Calibration	Total IgG calibration	1-point calibration
Calibrators	6 levels with assigned values of: 0, 4, 10, 20, 100, 600 µg/L	Not specified
Calibration Curve	Option to store the calibration curve for up to 28 days and run curve controls in each assay for calibration	Not specified
Internal Controls	Positive and Negative Control sera provided in a separate package	High Positive, Low Positive and Negative Control sera included in the assay kit
Reported Unit	EliA U/mL	U/mL
Reportable Range	0.6 – 297.1 EliA U/mL	Not specified
Limit of detection	0.6 U/mL	Not specified
Results Interpretation	Negative: <7.0 EliA U/mL Equivocal: 7.0 – 10.0 EliA U/mL Positive: >10.0 EliA U/mL	Negative: <20 Units Positive: 20 - 39 Units Positive: 40 - 80 Units Positive: > 80 Units

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

CSLI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

CLSI H18-A3: Procedures for the Handling and Processing of Blood Specimens; Approved Guideline

**L. Test Principle:**

The EliA™ Scl-70<sup>S</sup> Wells are coated with human recombinant Scl-70 protein. If present in the patient's specimen, antibodies to Scl-70 bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgA 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 value of fluorescent signal detected by the instrument, 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.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Seven serum samples selected from different parts of the claimed assay range were tested on Phadia® 100 and Phadia® 250 instruments to establish intra- and inter-assay precision. Each sample was run in 4 replicates for 7 days on 3 instruments. One batch was used to determine the precision of the assays on Phadia® 100 (equal to 84 replicate determinations per sample). Three batches were used to determine the precision of the assays on Phadia® 250 (equal to 252 replicate determinations per sample). Results summarized below met %CV specifications for both intra-assay (<10%) and inter-assay (<8%) imprecision.

<b>EliA™ Scl-70<sup>S</sup> on Phadia® 100 (n = 84)</b>						
Mean value (EliA U/mL)	Intra-assay		Inter-assay		Total Imprecision	
	SD	CV%	SD	CV%	SD	%CV
1.9	0.07	3.90	0.06	3.13	0.09	5.00
7.4	0.34	4.53	0.23	3.09	0.41	5.48
8.3	0.38	4.60	0.21	2.54	0.43	5.25
8.8	0.25	2.89	0.18	2.06	0.31	3.55
30.2	0.97	3.21	0.73	2.41	1.21	4.01
193.0	9.46	4.90	6.77	3.51	11.63	6.03
203.2	9.01	4.44	7.63	3.75	11.81	5.81

<b>EliA™ Scl-70<sup>S</sup> on Phadia® 250 (n = 252)</b>						
Mean value (EliA U/mL)	Intra-assay		Inter-assay		Total Imprecision	
	SD	CV%	SD	CV%	SD	%CV
2.5	0.07	3.02	0.07	2.71	0.10	4.06
7.5	0.23	3.08	0.09	1.25	0.25	3.33
8.2	0.22	2.69	0.18	2.21	0.29	3.48
10.8	0.30	2.80	0.21	1.99	0.37	3.44
28.2	0.76	2.71	0.64	2.26	0.99	3.53
191.6	8.42	4.39	7.17	3.74	11.06	5.77
200.1	8.91	4.46	6.11	3.05	10.81	5.40

The SD and %CV for lot-to-lot reproducibility on the Phadia® 250 ranged from 0.02 to 10.55 U/mL and 0.96% to 5.27%, respectively.

The SD and %CV for instrument-to-instrument variability on the Phadia® 100 ranged from 0.04 to 6.62 U/mL and 2.3% to 4.5%, respectively. On the Phadia® 250, the SD and %CV for instrument-to-instrument variability ranged from 0.04 to 8.02 U/mL and 0.5% to 4.0%, respectively.

*b. Linearity/assay reportable range:*

Five patient serum samples were serially diluted using EliA™ Sample Diluent and tested in 3 replicates in 1 run with 1 batch of EliA™ Scl-70<sup>S</sup> and one set of system reagents on the Phadia® 100 or Phadia® 250 instruments. The observed values were graphed against the calculated values and a linear regression was performed. Results are summarized below:

<b>EliA™ Scl-70<sup>S</sup> on Phadia® 100</b>					
Sample	Dilution range (EliA U/mL)	Slope (95% CI)	Intercept (95% CI)	R <sup>2</sup>	%CV Range
1	1.0 – 28.1	0.96 (0.94 to 0.97)	-0.10 (-0.26 to -0.06)	1.00	1.7 – 4.5
2	1.8 – 172.3	1.11 (1.10 to 1.12)	-0.90 (-1.46 to -0.345)	1.00	0.4 – 6.5
3	2.1 – 185.5	1.00 (0.98 to 1.01)	0.15 (-0.49 to 0.80)	1.00	0.7 – 3.4
4	2.1 – 192.0	1.06 (1.03 to 1.09)	0.30 (-1.32 to 1.92)	1.00	0.9 – 9.7
5	2.9 – 297.1	1.10 (1.06 to 1.14)	-1.13 (-4.06 to 1.79)	1.00	1.0 – 7.4

EliA™ Scl-70 <sup>S</sup> on Phadia® 250					
Sample	Dilution range (EliA U/mL)	Slope (95% CI)	Intercept (95% CI)	R <sup>2</sup>	%CV Range
1	0.7 – 24.3	1.04 (1.01 to 1.07)	0.08 (-0.13 to 0.29)	1.00	0.8 – 58.2
2	1.4 – 159.9	1.09 (1.06 to 1.11)	0.66 (-0.40 to 1.71)	1.00	1.0 – 12.1
3	1.9 – 207.7	0.96 (0.95 to 0.97)	-0.40 (-1.11 to 0.31)	1.00	1.3 – 9.0
4	2.1 – 193.6	1.06 (1.04 to 1.09)	0.05 (-1.15 to 1.25)	1.00	0.3 – 8.7
5	3.1 – 298.9	1.09 (1.05 to 1.13)	0.65 (-2.73 to 4.04)	1.00	0.1 -3.1

The technical measuring range (detection limit, upper limit) for EliA Scl-70<sup>S</sup> is from 0.6 to  $\geq 240$  EliA U/mL. The upper limit of the reported results in EliA U/mL can vary due to a lot-specific conversion from  $\mu\text{g/L}$  to EliA U/mL. Results above the upper limit are reported as “above”. Linearity was shown for samples in the range from 0.6 to 297.1 EliA U/mL. The labeling states that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the technical measuring range.

High dose hook effect: A hook effect was not observed when analyzing a high positive serum sample with an estimated concentration more than 14 times above the upper limit of the technical measuring range.

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

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

Traceability – The IgG calibrators are traceable (via unbroken chain of calibrations) to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from the World Health Organization (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.

There is no international standard for IgG antibodies directed to Scl-70<sup>S</sup>. The instrument measures specific IgG concentration in  $\mu\text{g/L}$  which is automatically converted to EliA U/mL by using a lot-specific conversion factor given by the bar code printed on the EliA™ Scl-70<sup>S</sup> Well package and read by the instrument during reagent loading.

Calibrators – The calibrators are human serum in standard buffer. There are 6 levels with assigned values from 0-600  $\mu\text{g/mL}$ . The calibrator curve is acquired by fitting the values of the 6 calibrators and can be stored for up to 28 days by the instrument to be used on additional assays. Each EliA™ Scl-70<sup>S</sup> assay includes calibrator (curve) controls that have to recover in defined ranges to ensure that the stored calibration curve is still valid.

Controls – The EliA™ ANA Positive Control and EliA™ IgG/IgM/IgA Negative Control were cleared under k072393 and k091845, respectively. 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. EliA IgG/IgM/IgA Negative Control is prepared from selected pooled sera from normal, healthy donors. The controls are prediluted and ready for use. Each EliA™ Control package contains a Control Certificate listing predefined acceptance criteria for the EliA™ products the Controls can be used with. The target ranges of the EliA™ Controls tested with the EliA Scl-70 assay on the two Phadia® instruments are summarized below:

<b>Instrument</b>	<b>EliA™ ANA Positive Control</b>
Phadia® 100	86.4 – 201.7 EliA U/mL
Phadia® 250	78.6 – 183.4 EliA U/mL
<b>Instrument</b>	<b>EliA™ IgG/IgM/IgA Negative Control</b>
Phadia® 100	< 6 EliA U/mL
Phadia® 250	< 6EliA U/mL

ii) *Kit Stability:*

Shelf-life stability – An accelerated stability study set the shelf-life stability of the EliA™ Scl-70<sup>S</sup> Wells (from the date of manufacture when stored at recommended temperature 2-8°C) at 24 months. A real-time stability study is underway and currently supports a 7-month stability claim. All studies were performed on three lots of EliA Scl-70<sup>S</sup> Wells. Other required components (previously reviewed) of the test method have a shelf life of 18 - 24 months. The sponsor notes that it is important to store the wells in dry conditions at 2-8°C.

Open stability – An accelerated study set the stability of the foil bag containing the EliA™ Scl-70<sup>S</sup> Wells after first opening at 9 months at 2-8°C. A real-time stability study supported the 9-month stability claim. The stability after first opening study for EliA™ IgG Calibrators and EliA™ IgG Curve Control are not required as they are for single use only.

On-board stability – The on-board stability of the EliA™ Scl-70<sup>S</sup> Wells packed in carrier storage tray without desiccant bag was tested 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 EliA™ Scl-70<sup>S</sup> Wells can be stored open at 2-8°C for up to 28 days.

iii) *Sample Storage:*

The package insert 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 are not completed within eight hours, serum/plasma should be refrigerated (2- 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 (LoQ) were determined in accordance with CLSI EP17-A. On each Phadia instrument (Phadia® 100 and Phadia® 250), a blank sample (for LoB) and five low antibody samples (for LoD and LoQ) were assayed in 12 replicates in each of six runs on different days, three runs in two

instruments, for a total 72 replicates per sample. The total error for the LoD was estimated to be lower than the target level in both instruments. The LoQ was set to the same value as the LoD. The values determined are presented in the table below:

EliA™ Scl-70 <sup>S</sup>	LoB	LoD	LoQ
Phadia 100	0.25 EliA U/mL	0.36 EliA U/mL	0.36 EliA U/mL
Phadia 250	0.31 EliA U/mL	0.52 EliA U/mL	0.52 EliA U/mL

e. *Analytical specificity:*

i) *Endogenous Interference:*

Interferences were assessed by testing five samples (two negative samples, two samples within the equivocal range and a low positive sample). Each sample was spiked with the interfering substances or substance-specific blanks, and analyzed in two runs, three replicates each (n=6), on one lot of EliA™ Scl-70<sup>S</sup> Well and one lot of system reagents. The data demonstrated that EliA™ Scl-70<sup>S</sup> was not adversely affected (percent recovery ranged from 91 to 100%) by the following substances up to the concentrations listed in the table below:

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

The package insert contains a caution not to use hemolyzed, lipemic, or icteric samples.

ii) *Cross-reactivity:*

Cross reactivity was investigated using international reference sera from AMLI (Association of Medical Laboratory Immunologists) and CDC samples (Center of Disease Control and Prevention). Each sample was assayed in duplicates using one batch of EliA™ Scl-70<sup>S</sup> Wells and one batch of system reagents. The 10 AMLI samples including the AMLI sample 6 which is defined as Scl-70 specific by AMLI were all negative. The sponsor stated that this sample has never shown any reactivity for Scl-70 on VareliSA Scl-70, VareliSA ReCombi ANA Profile, and INOVA QuantaLite ANA. One of the 12 CDC samples contains Scl-70-specific antibodies and was found positive on EliA™ Scl-70<sup>S</sup>.

f. *Assay cut-off:*

Based on the results of the expected values/reference range study described below in Section M.5, the 99<sup>th</sup> percentile lies below the upper limit of the equivocal range for EliA™ Scl-70<sup>S</sup>. The assay cutoffs were set as follows:

Decision point	Interpretation
<7.0 U/mL	Negative
7.0 – 10.0 U/mL	Equivocal
>10.0 U/mL	Positive

In case of equivocal results, it is recommended to retest the patient after 8-12 weeks.

## 2. Comparison studies:

### a. *Method comparison with predicate device:*

For analysis of agreement with a predicate device, a total of 336 clinically defined serum samples were collected from the serum bank at Phadia GmbH and assayed in singleton on both QuantaLite™ Scl-70 ELISA and EliA™ Scl-70<sup>S</sup>. The clinically defined samples were from patients diagnosed with Scleroderma (n = 101), limited cutaneous form of systemic sclerosis (n = 33), mixed connective tissue disease (n = 37), Systemic lupus erythematosus (n = 34), Sjögren's syndrome (n = 26), Poly/Dermatomyositis (n = 5), Rheumatoid arthritis (n = 30), various cancers (n = 20), various bacterial infections (n = 24) and various viral infections (n = 26). In addition, 54 samples with known anti-EliA Scl-70<sup>S</sup> reactivity but without clinical diagnosis were added to the analysis. Of the 390 sera tested, 16 results were excluded as being outside of the measuring range of the EliA™ Scl-70<sup>S</sup> assay and the predicate. The results are summarized below:

		QuantaLite™ Scl-70 ELISA (U/mL)		Total
		Positive: >20	Negative: ≤20	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >10.0	51	9	60
	Equivocal: 7.0 – 10.0	2	12	14
	Negative: <7.0	4	296	300
Total		57	317	374

Agreements were calculated by grouping EliA™ Scl-70<sup>S</sup> equivocal results with its test negative results, and then agreements were calculated again by grouping EliA™ Scl-70<sup>S</sup> equivocal results with the test positive results:

Equivocal EliA™ Scl-70 <sup>S</sup> results considered as negative		QuantaLite™ Scl-70 ELISA (U/mL)		Total
		Positive: >20	Negative: ≤20	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >10.0	51	9	60
	Negative: ≤10.0	6	308	314
Total		57	317	374

Positive percent agreement:	89.5% (51/57)	95% CI: 77.8 – 95.6%
Negative percent agreement:	97.2% (308/317)	95% CI: 94.5 – 98.6%
Total percent agreement:	95.9% (359/374)	95% CI : 93.3 – 97.7%

Equivocal EliA™ Scl-70 <sup>S</sup> results considered as positive		QuantaLite™ Scl-70 ELISA (U/mL)		Total
		Positive: >20	Negative: ≤20	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >7.0	53	21	74
	Negative: ≤7.0	4	296	300
Total		57	317	374

Positive percent agreement: 93.0% (53/57) 95% CI: 82.2 – 97.8%  
 Negative percent agreement: 93.4% (296/317) 95% CI: 89.9 – 95.8%  
 Total percent agreement: 93.3% (349/374) 95% CI: 90.2 – 95.5%

*b. Matrix comparison:*

A study was performed to demonstrate that heparin plasma and EDTA plasma matrices yield comparable values as serum in the EliA™ Scl-70<sup>S</sup> immunoassay. A total of 52 matrix-matched samples spread across the reportable range were assayed once on the Phadia® 250 instrument. Negative samples did not switch to positive in any serum/plasma combination. A Passing & Bablok analysis was performed and the results are depicted in the following table:

	Range tested (EliA U/mL)	Slope (95% CI)	Intercept (95% CI)	R <sup>2</sup>
<b>Serum vs. EDTA plasma</b>	0.6 – 264.2	1.00 (0.99 – 1.02)	-0.05 (-0.29 – 0.21)	1.00
<b>Serum vs. Heparin plasma</b>	0.6 – 278.9	1.05 (1.03 – 1.07)	-0.08 (-0.24 – 0.17)	1.00

*c. Instrument comparison:*

A study was performed to demonstrate that the performance of EliA™ Scl-70<sup>S</sup> was equivalent on the Phadia® 100 and Phadia® 250 instruments. A total of 36 samples (24 positive, 8 equivocal and 4 negative) spanning the assay range were analyzed in 6 runs in single replicates on 3 Phadia®100 and 3 Phadia® 250 instruments, with 2 runs 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 Scl-70 <sup>S</sup> Weighted Deming Regression: Phadia®100 vs. Phadia® 250		
Instrument Pair	Slope (95% CI)	Intercept (95% CI)
Phadia®100 #1 vs. Phadia® 250 #1	0.94 (0.90 – 0.98)	0.05 (-0.13 – 0.20)
Phadia®100 #2 vs. Phadia® 250 #2	0.97 (0.94 – 1.01)	-0.09 (-0.26 – 0.07)
Phadia®100 #3 vs. Phadia® 250 #3	1.02 (0.98 – 1.06)	0.19 (-0.08 – 0.47)

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

The performance of EliA™ Scl-70<sup>S</sup> was compared to a clinical diagnosis of Scleroderma. A total of 101 samples from individuals diagnosed with the diffuse form of scleroderma, 33 samples from individuals with CREST and 202 samples from individuals with other disease conditions were tested on the Phadia® 250 instrument.

		Diagnosis		Total
		Positive	Negative	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >10.0	31	3*	34
	Equivocal: 7.0-10.0	0	1	1
	Negative: < 7.0	70	231	301
Total		101	235	336

\* 1 Sjögren's syndrome and 2 CREST

Clinical sensitivity: 30.7% (31/101)      95% CI: 22.1 – 40.8%

Clinical specificity: 98.7% (234/235)      95% CI: 96.0 – 99.7%

CREST syndrome is the limited cutaneous form of systemic sclerosis associated with antibodies against centromeres. At the beginning of the disease, a differentiation between the limited and the diffuse cutaneous form is difficult, and patients may be incorrectly classified. The clinical performance evaluation was performed first without, and then with, the 33 samples with CREST diagnosis. In both evaluations, the clinical sensitivity and specificity were calculated by grouping the assay's equivocal results with its test negative results, and then sensitivity and specificity were calculated again by grouping the assay's equivocal results with its test positive results.

Clinical performance evaluation that includes the 33 samples with CREST diagnosis and that considers the equivocal samples as negative is summarized in the following table:

Equivocal EliA™ Scl-70 <sup>S</sup> results considered as negative		Diagnosis		Total
		Positive	Negative	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >10.0	31	3	34
	Negative: ≤10.0	70	232	302
Total		101	235	336

Clinical sensitivity: 30.7% (31/101)      95% CI: 22.1 – 40.8%

Clinical specificity: 98.7% (232/235)      95% CI: 96.0 – 99.7%

Clinical performance evaluation that includes the 33 samples with CREST diagnosis and that considers the equivocal samples as positive is summarized in the following table:

Equivocal EliA™ Scl-70 <sup>S</sup> results considered as positive		Diagnosis		Total
		Positive	Negative	
EliA™ Scl-70 <sup>S</sup> (EliA U/mL)	Positive: >7.0	31	4	35
	Negative: ≤7.0	70	231	301
Total		101	235	336

Clinical sensitivity: 30.7% (31/101) 95% CI: 22.1 – 40.8%

Clinical specificity: 98.3% (231/235) 95% CI: 95.4 – 99.4%

The performance on samples from individuals diagnosed with other disease conditions is summarized below. Equivocal results evaluated as negative:

Diagnostic groups	N	No (%) Positive QuantaLite™ Scl-70 ELISA	No (%) Positive EliA™ Scl-70 <sup>S</sup>
CREST	33	2 (6%)	2 (6%)
Mixed connective tissue disease	37	0 (0%)	0 (0%)
Systemic lupus erythematosus	34	1 (3%)	0 (0%)
Sjögren's syndrome	26	1 (4%)	1 (4%)
Poly/Dermatomyositis	5	0 (0%)	0 (0%)
Rheumatoid arthritis	30	0 (0%)	0 (0%)
Cancers*	20	0 (0%)	0 (0%)
Bacterial infections**	24	0 (0%)	0 (0%)
Viral infections***	26	2 (4%)	0 (0%)

\*17 Breast cancer, 2 rectal cancer and 1 kidney cancer

\*\*20 Borrelia and 4 *Helicobacter pylori*

\*\*\*5 Epstein-Barr virus, 8 Hepatitis B virus, 6 Hepatitis C virus, 2 Norovirus and 5 others

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:

A total of 400 apparently healthy blood donor samples from a Caucasian population equally distributed by gender and age were measured on the Phadia® 250 instrument.

The results are summarized below:

	EliA U/mL
Mean	0.8
Median	0.7
Range	0.2 – 11.1
95 <sup>th</sup> percentile	1.4
99 <sup>th</sup> percentile	2.4

The proportion of sera tested positive by EliA™ Scl-70<sup>S</sup> is below 1%. None of the samples fell in the equivocal range. No significant difference between male and female was observed. The Scl-70<sup>S</sup> concentration is not normally distributed and ranges from 0.3 EliA U/mL (2.5<sup>th</sup> percentile) to 1.7 EliA U/mL (97.5<sup>th</sup> percentile). Expected values may vary depending on the population tested.

**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.