

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

A. 510(k) Number:

k113143

B. Purpose for Submission:

New device

C. Measurand:

Anti-Scl-70 (topoisomerase-1) antibodies

D. Type of Test:

Qualitative and semi-quantitative

E. Applicant:

IMMCO Diagnostics Inc.

F. Proprietary and Established Names:

ImmuLisa Scl-70 Antibody ELISA

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5100, Antinuclear Antibody Immunological Test System

2. Classification:

Class II

3. Product code:

LLL, Extractable Antinuclear Antibody, Antigen and Control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

An enzyme-linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Scl-70 antibodies in human serum, as an aid in the diagnosis of systemic sclerosis /scleroderma in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

An ELISA Microplate reader capable of reading absorbance values at 450nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650nm. Also required is an automatic microplate washer capable of accurately dispensing 200 µl of fluid.

I. Device Description:

The kit contains: five levels of calibrators (1 EU/ml, 20 EU/ml, 40 EU/ml, 80 EU/ml, and 160 EU/ml), a microplate coated with Scl-70 antigen, a positive control, a negative control, horseradish peroxidase conjugated goat anti-human IgG, serum diluent, wash buffer concentrate, TMB, and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s) and number:
Diamedix Scl-70 Antibody ELISA (k970239)
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	An enzyme-linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Scl-70 antibodies in human serum	Same
Methodology	ELISA	Same
Detection of antibodies	Scl-70 IgG	Same
Cutoff	20 EU/mL	same

Differences		
Item	Device	Predicate
Intended Use	Aid in the diagnosis of systemic sclerosis / scleroderma in conjunction with other laboratory and clinical findings	Aid in the diagnosis of autoimmune disease
Calibrators	Five (5) point calibrator curve	Single calibrator
Reportable Range	1.5 – 160 EU/mL	Not specified
Limit of Detection	1.5 EU/mL	Not specified
Conjugate	Horseradish peroxidase (HRP)	Alkaline Phosphatase
Substrate	3,3',5,5' tetramethylbenzidine (TMB)	Para-Nitrophenylphosphate (pNPP)

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

CLSI EP-17A “Protocols for Determination of Limits of Detection and Limits of Quantitation”

L. Test Principle:

Calibrators, controls, and diluted patient samples are added to the wells and autoantibodies recognizing the Scl-70 antigen bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured human autoantibody. Excess unbound conjugate is removed by another wash step. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate. The intensity of color development is proportional to the concentration of autoantibody in the sample. Microtiter plates are read at 450 nm. In the qualitative protocol, the controls and patient results are determined by calculating the ratio of the sample OD to the cut-off calibrator OD and reported as positive if the sample OD \geq calibrator OD. In the semi-quantitative protocol, a 5-point calibration curve is used to calculate the sample result in EU/mL; values between 20 and 25 EU/mL are considered indeterminate (borderline). The sponsor recommends that indeterminate samples be retested.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Semi-quantitative precision:

Six samples from different parts of the claimed assay range were tested to establish between-day precision and within-run repeatability. Between-day imprecision was tested in replicates of six over 13 assay runs (n = 78); Within run repeatability was tested by twelve replicates performed in a single run. The total number of replicates was 90.

Scl-70 Sample	Mean	Within Run		Between Days		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV
1	4.8	0.43	8.4	0.41	8.8	0.43	9.0
2	19.7	1.20	6.2	0.96	4.8	0.99	5.0
3	23.9	0.99	4.4	1.53	6.3	1.55	6.5
4	68.6	3.33	4.9	5.98	8.7	5.69	8.3
5	122.1	8.44	7.0	10.38	8.5	10.11	8.3
6	149.3	3.43	2.3	8.73	5.8	8.21	5.5

Qualitative Repeatability:

Four samples were tested in 90 runs; a low negative sample (but above the Limit of Detection), a sample $\leq 20\%$ below the cut-off of 20 EU/mL, a sample $\leq 20\%$ above the cut-off, and a moderate positive.

Sample	Mean (EU/mL)	Expected Result	% Expected Result
1	3.8	Negative	100
2	17.7	Negative	100
3	22.8	Positive	100
4	55.4	Positive	100

b. *Linearity/assay reportable range:*

Three serum samples were selected to cover the entire range of the assay. Each sample was proportionally diluted with a known negative serum sample and tested. The observed values were graphed against the calculated values and linear regression was performed. The study results are summarized in the table below:

Sample	Test Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	%Recovery
1	3.1 – 39.1	0.90 (0.75 to 1.05)	0.46 (-3.12 to 4.03)	0.97	84 to 118
2	3.2 – 123.9	0.92 (0.78 to 1.06)	-1.48 (-11.89 to 8.93)	0.98	83 to 103
3	3.5 – 149.8	0.94 (0.86 to 1.02)	1.33 (-6.0 to 8.75)	0.99	91 to 113

The claimed reportable range of the assay was determined to be from the 1.5 to 160 EU/mL.

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

Traceability: There is no reference standard for anti-Scl-70 antibodies. Positive control and calibrators were derived from different lots of the commercially available sera of systemic sclerosis patients positive for Scl-70 antibody. Antibody positivity and concentrations are confirmed using commercially available assays for Scl-70 antibodies. As new lots of calibrators are developed, comparison studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested in comparison with a panel of internal control samples. For assignment of values, the samples were tested at various dilutions on at least two different lots of Scl-70 coated plates.

Kit stability: Real time stability studies support a claim of 18 months of an unopened device. The manufacturer demonstrated that opened kits were stable for 30 days when stored at the recommended conditions (2°-8°C). The sponsor recommends resealing unused plates in their envelope and closing and returning reagents to appropriate storage immediately after use.

Sample Stability: The sponsor presented a study that supports a claim that serum samples are stable at 2°- 8°C for one week.

d. *Detection limit:*

The limit of blank (LoB) and limit of detection (LoD) were determined by following the study design recommended in CLSI EP17-A. Sixty replicates of the kit diluent were run; the LoB was determined sorting the results from low to high by OD and averaging the value of blanks in the 57th and 58th positions. The LoB was calculated in EU/ml by comparing this average to the standard curve; the resulting LoB was 1.4 EU/mL.

To determine the Limit of Detection (LoD), six normal serum samples were tested ten times each (a total of 60 determinations). These samples were used to calculate LoD according to CLSI EP17-A. The LoD of the assay is 1.5 EU/mL.

e. *Analytical specificity:*

Ten CDC ANA human reference sera from the Centers for Disease Control and Prevention were tested with the Immulisa Scl-70 Antibody ELISA. As expected, the CDC sample known to contain anti-Scl-70 antibodies tested strongly positive. The nine other samples were negative. The other samples represent other ANA-type antigens such as SS-A, SS-B, Jo-1, etc.

Endogenous interferences: The following substances were spiked into serum in order to test for interference: hemoglobin (2 g/L), bilirubin (342 µmol/L), and rheumatoid factor (RF, 100 IU/mL). Five samples were evaluated for interference – a negative above the LoD, two samples just below the cut-off, and two strongly positive samples. Recoveries were all within ± 15% for all sample/substance combinations.

In a separate study the interference by triglycerides (37 mmol /L) and

cholesterol (13 mmol/L) were investigated in a similar panel of samples. Recoveries were all within $\pm 10\%$ for all sample/substance combinations. The instructions for use caution against the use of lipemic, hemolyzed, or bacterially contaminated samples.

f. Assay cut-off:

The cut-off was assigned a unit value of 20 EU/mL based on the standardized method used by other IMMCO products.

2. Comparison studies:

a. Method comparison with predicate device:

The subject device was evaluated by testing well-characterized sera of systemic sclerosis /scleroderma (SSc) subjects (n = 63) and non-scleroderma disease controls (n = 44). These specimens were also tested on the predicate device using the same testing conditions and environment. Only samples in the reportable range of the subject device were included in this analysis.

Indeterminate (20 – 25 EU/mL) samples considered assay positive:

		Predicate		
		Positive	Negative	Total
IMMCO Scl-70 ELISA	Positive	54	7	61
	Negative	1	45	46
	Total	55	52	107

Positive % Agreement: 98.2% (95% CI: 89.0% - 99.9%)

Negative % Agreement: 86.5% (95% CI: 73.6% - 94.0%)

Overall % Agreement: 92.5% (95% CI: 85.4% - 96.5%)

Indeterminate (20 – 25 EU/mL) samples considered assay negative:

		Predicate		
		Positive	Negative	Total
IMMCO Scl-70 ELISA	Positive	50	8	58
	Negative	3	46	49
	Total	53	54	107

Positive % Agreement: 94.3% (95% CI: 83.4% – 98.5%)

Negative % Agreement: 85.2% (95% CI: 72.3% – 92.9%)

Overall % Agreement: 89.7% (95% CI: 82.0% – 94.5%)

Qualitative analysis: ≥ 20 EU/mL considered positive

		Predicate		
		Positive	Negative	Total
IMMCO Scl-70 ELISA	Positive	54	7	61
	Negative	1	45	46
	Total	55	52	107

Positive % Agreement: 98.2% (95% CI: 89.0% - 99.9%)

Negative % Agreement: 86.5% (95% CI: 73.6% - 94.0%)

Overall % Agreement: 92.5% (95% CI: 85.4% - 96.5%)

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

The clinical sensitivity and specificity of a set of 373 clinically characterized sera (see below for diagnoses) was evaluated with the assay. The samples were obtained from clinical investigators, left-over reference laboratory specimens, and from commercial sources. The results of this testing are shown in the tables below:

Indeterminate Samples (samples 20 – 25 EU/mL) considered positive:

		Systemic Sclerosis Diagnosis		
		Positive	Negative	Total
IMMCO Scl-70 ELISA	Positive	67	6	73
	Negative	106	194	300
	Total	173	200	373

Sensitivity (67/173) = 38.7% (95% C.I. = 31.5 – 46.4%)

Specificity (194/200) = 97.0% (95% C.I. = 93.3 – 98.8%)

Indeterminate Samples (samples 20 – 25 EU/mL) considered negative:

		Systemic Sclerosis Diagnosis		
		Positive	Negative	Total
IMMCO Scl-70 ELISA	Positive	62	6	68
	Negative	111	194	305
	Total	173	200	373

Sensitivity (62/173) = 35.8% (95% C.I. = 28.8 – 43.5%)

Specificity (194/200) = 97.0% (95% C.I. = 93.3 – 98.8%)

Anti-Scl-70 Assay Results by Clinical Diagnosis:

Patient Group	n =	n positive*	% Positive
Systemic Sclerosis/Scleroderma	173	67	38.7 %
Limited SSc	47	12	25.5%
Diffuse SSc	29	17	58.6%
Subtype not identified	97	38	39.2%
Celiac Disease	6	0	0
Polymyositis/Dermatomyositis	132	5	3.8%
Rheumatoid Arthritis	7	0	0
Sjorgen’s Syndrome	17	0	0
Systemic Lupus Erythematosus	22	1	4.5%

Patient Group	n =	n positive*	% Positive
Hashimoto's Thyroiditis	8	0	0
Autoimmune Vasculitis	8	0	0
Total non-normal	373	73	

* = borderline considered positive

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The expected value in the general population is negative. A study of 133 normal, apparently disease-free samples showed two yielded borderline results (1.5%).

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.