CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Scl-70 (Scleroderma Antibody)
- New Test Introduction –

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Ordering Information:

Mnemonics:	**Scl-70 IgG Ab**
Test Name:	Scl-70 IgG Ab
Test Number:	3000826
Specimen:	1 ml serum, refrigerated up to 48 hours, frozen up to 30 days

Effective Date: Testing will begin on Monday, April 6, 2015.

Performed: Monday, Wednesday, and Friday

Reference Range: Negative

Background:
The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of connective tissue diseases. Antibodies against Scl-70 are characteristic and specific for scleroderma (particularly the diffuse form; frequency up to 70%).

EliA Scl-70^S 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 scleroderma (diffuse form) in conjunction with other laboratory and clinical findings. EliA Scl-70^S uses the EliA IgG method on the instrument Phadia 250.

Principle of Test:
The EliA Scl-70^S 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 IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, nonbound 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 assay directly measures the amount of antibody of interest bound to the antigen coating the EliA well, therefore 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.

Results Interpretation:
Phadia 250 measures specific IgG concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA EliA Scl-70^S Well, the results are automatically converted to EliA U/ml. There are no international standards for the Scl-70^S antibodies. The manufacturer's unit of EliA U/ml is arbitrary and will be reported as U/ml.

Negative: <7 U/ml
Equivocal: 7-10 U/ml
Positive: >10 U/ml

The measuring range (detection limit, upper limit) for EliA EliA Scl-70^S is from 0.6 to ≥ 240 U/ml. Results less than 0.6 will be reported as <0.6 and results greater than 240 will be reported as ≥ 240.

Limitations:
1. A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.

Validation Data:
Precision:
To assess within-run precision, EliA Negative and EliA ANA Positive controls were run in the same run ten times each. To assess between-run precision, EliA Negative and EliA ANA Positive controls were run ten times each on two different days. Performance was within manufacturer's precision claims of <10% CV. (Table 1)

Table 1: EliA Scl-70 Precision

Phadia SN	Within Run Precision				Between Run Precision			
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
N01926	< 0.6 U/mL	0%	122.0 U/mL	2.19%	< 0.6 U/mL	0%	130.5 U/mL	4.96
N01778	< 0.6 U/mL	0%	128.7 U/mL	5.54%	< 0.6 U/mL	0%	145.7 U/mL	5.54

Method Comparison
A total of 49 specimens were split and processed utilizing Phadia 250 EliA Scl-70^s assay. The specimens were run on both Phadia 250 instruments. There is a 100% negative agreement for the two assays between the two Phadia 250 instruments and the Quest assay. Three out of twenty-four specimens that Quest reported as positive, were positive by the Phadia 250 Scl-70^s assay. The other twenty-one specimens were negative by the Phadia assay. Positive agreement is 12.5%. The results between the two Phadia instruments showed 100% agreement.

A poster submitted by the Medical University of Vienna compared the BioPlex2200 (Quest's method) and the ImmunoCAP 250 (Phadia) assays for Scl-70. Fourteen individuals were positive on the BioPlex2200 system, 10 of which could not be confirmed by other reference methods (71%). One of five positive specimens on the Phadia system could not be confirmed (20%). The conclusion by the Medical University of Vienna was the ImmunoCAP 250 based on EliA technology provided the most reliable results concerning sensitivity and specificity, whereas tests based upon the Luminex system (BioPlex2200) seemed to be challenged by a considerable number of false positive results.

To confirm these findings, CPAL tested nine of the discordant results with differing Quest values by IFA using ImmunoConcepts' HEp-2000 IgG Fluorescent ANA-Ro Test System. Three of the nine specimens were negative by IFA. The other six specimens were positive with patterns that were either speckled, homogenous, or nucleolar. The patterns did not resemble the Scl-70 pattern, confirming that these were false positive by the Quest method.

References:

1. EliA Scl-70^S package insert, October 2014.