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Anti – Scl70
Qualitative
Assay Development Report

Theranos, Inc

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1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications[TC "Assay Specifications" \f C \l "3"]

This assay is designed to qualitatively determine anti-Scl70 antibodies in human plasma and serum using sandwich ELISA.

1.1.1 Reference Assays [TC "Reference Assays and Standards" \f C \l "3"]

The following commercial ELISA kits have been used in house as predicate methods:

- INOVA QUANTA Lite Scl-70 ELISA (Cat# 708580)
- Immco Diagnostics ImmuLisa™ Scl-70 Antibody ELISA (Cat# 5150)
- IBL Scl-70 Antibody ELISA (Cat# 75251)

1.1.2 Materials and Methods[TC "Materials and Methods" \f C \l "1"]

An Scl-70 antigen coated surface serves as the capture surface for the Scl-70 assay. The sample (plasma or serum) is diluted and incubated on the capture surface. The surface is then washed. An alkaline phosphatase (AP)-labeled anti-human IgG antibody is then incubated on the surface. After the detection antibody incubation, another washing cycle is performed and the alkaline phosphatase substrate is incubated, and the resulting chemiluminescence is read in Relative Light Units (RLU).

1.2 Normal Sample Screen: Cut-Off Determination

Fifteen (15) randomly obtained normal donor serum samples were obtained from Stanford and screened on the Theranos system to determine the cutoff value. The Theranos cutoff value was determined by taking the mean RLU of the 15 normal samples plus 5 times the standard deviation. It is imperative that normal samples used for cutoff determination include samples with both low and high signals in order to effectively reproduce a more reliable cutoff value. The sample RLU divided by the cutoff value yields its Antibody Index. Samples are considered to be positive, borderline, or negative for Scl-70 antibodies if their Ab Indices are found to be greater than 1.1, between 0.9 and 1.1, or less than 0.9, respectively.

Ab Index > 1.1
Ab Index > 0.9, < 1.1
Ab Index < 0.9

The same 15 samples were also screened using the predicate methods listed in section 1.1.1. The Theranos results correlated very well with INOVA ELISA kit's results. All 15 samples tested negative on both platforms. However, there were samples that were detected as positive on the other two commercial kits which were tested negative on the Theranos system. This discrepancy was probably a result of repeated sample thawing/freezing which most likely had a negative

impact on sample stability. In general, there was excellent correlation between Theranos results and those obtained from the predicate methods listed in section 1.1.1. Results are summarized in Table 1.

Table [SEQ Table * ARABIC]. Normal Sample Screen: Cut-Off Determination

Sample ID	Matrix	Intra-Cartridge		Inter-Cartridge		ANTIBODY INDEX			
		Tip 1	Tip 2	Mean	%CV	Theranos	INOVA	IMMCO	IBL
CLN1	Serum	4284	4784	4458	8	0.05	4	5	0.15
		-	-						
		4711	4055						
CLN2	Serum	4869	-	4042	12	0.04	4	2	0.07
		3594	3835						
		4052	3862						
CLN3	Serum	4791	6179	5364	13	0.06	4	6	0.16
		5281	4280						
		5835	5817						
CLN4	Serum	8467	7082	8623	11	0.10	4	2	0.13
		8102	9423						
		9571	9095						
CLN5	Serum	7956	6826	6897	13	0.08	5	3	0.10
		6716	7712						
		5432	6740						
CLN6	Serum	5489	5697	6642	14	0.07	3	4	0.16
		7113	7228						
		6520	7803						

Note: Table 1 continues on next page.

Table 1 (Continued): Normal Sample Screen: Cut-Off Determination

Sample ID	Matrix	Intra-Cartridge		Inter-Cartridge		ANTIBODY INDEX			
		Tip 1	Tip 2	Mean	%CV	Theranos	INOVA	IMMCO	IBL
CLN7	Serum	5231	4427	4354	19	0.05	4	7	0.3
		5320	4290						
		3141	3715						
CLN8	Serum	9039	9761	9669	19	0.11	5	5	0.15
		6967	6265						
		12751	10230						
CLN9	Serum	4299	4216	4655	12	0.05	3	14	0.08
		4377	4315						
		5192	5533						
CLN10	Serum	6337	4702	5013	15	0.06	3	6	0.12
		4559	5287						
		4987	4207						
CLN11	Serum	65871	64891	67877	9	0.75	9	33	0.29
		63263	68407						
		79344	65489						
CLN12	Serum	3666	3746	3392	11	0.04	5	5	0.22
		3075	3411						
		3619	2836						
CLN13	Serum	3925	4616	4252	8	0.05	4	2	0.18
		3986	4760						
		4198	4027						
CLN14	Serum	6772	6661	5744	14	0.06	11	174	3.06
		5053	4926						
		5537	5512						
CLN15	Serum	11911	12593	11972	8	0.13	4	N/A	0.28
		10291	11900						
		13126	12012						
Overall MEAN				10400					
Overall STDEV				15986					
CUT OFF				90330					

1.3 Clinical Sample Correlation

A total of 72 randomly obtained clinical serum samples from Scleroderma, Lupus and Sjogren's syndrome patients were screened on Theranos system. Data is compared to those provided from screening the same set of samples via three commercial ELISA kits that are specific for the detection of Scl-70 antibody.

The commercial ELISA kits were obtained from three different vendors: INOVA Diagnostics, IMMCO Diagnostics and IBL International. 72 normal and clinical sera obtained from Bioreclamation were collectively screened on all three kits. Samples that tested positive on any of the commercial kits were screened on the Theranos system to assess clinical correlation. There was generally good agreement among the Theranos Anti Scl-70 assay and the commercial ELISA kits' data. Results are reported in Table 2.

Table [SEQ Table * ARABIC]. Clinical Correlation Data

Sample ID	Human Test Samples			Inter-Cartridge		ANTIBODY INDEX			
	Matrix	Species	Strain	Mean	%CV	Theranos	INOVA	IMMCO	IBL
CLN1	Serum	Normal	N/A	3970	26	0.04	4	5	0.15
CLN2	Serum	Normal	N/A	3497	17	0.04	4	2	0.07
CLN3	Serum	Normal	N/A	5348	9	0.06	4	6	0.16
CLN4	Serum	Normal	N/A	8140	22	0.09	4	2	0.13
CLN5	Serum	Normal	N/A	6259	8	0.07	5	3	0.10
CLN6	Serum	Normal	N/A	5763	17	0.06	3	4	0.16
CLN7	Serum	Normal	N/A	4396	16	0.05	4	7	0.3
CLN8	Serum	Normal	N/A	9612	17	0.11	5	5	0.15
CLN9	Serum	Normal	N/A	5041	35	0.06	3	14	0.08
CLN10	Serum	Normal	N/A	4656	29	0.05	3	6	0.12
CLN11	Serum	Normal	N/A	52904	22	0.59	9	33	0.29
CLN12	Serum	Normal	N/A	4049	25	0.04	5	5	0.22
CLN13	Serum	Normal	N/A	4621	8	0.05	4	2	0.18
CLN14	Serum	Normal	N/A	3911	28	0.04	11	174	3.06
CLS1	Serum	Autoimmune	Scleroderma	663487	37	7.35	9	186	0.33
CLS2	Serum	Autoimmune	Scleroderma	65172	18	0.72	19	70	0.73
CLS9	Serum	Autoimmune	Scleroderma	144646	13	1.60	100	213	3.76
SS10	Serum	Autoimmune	Sjogren	91785	24	1.02	5	91	0.19
SCL06	Serum	Autoimmune	Scleroderma	85830	12	0.95	23	83	1.78
SCL16	Serum	Autoimmune	Scleroderma	573226	8	6.35	3	194	3.07
SCL25	Serum	Autoimmune	Scleroderma	324022	11	3.59	141	267	5.25
SCL29	Serum	Autoimmune	Scleroderma	274788	27	3.04	124	257	2.66
SCL38	Serum	Autoimmune	Scleroderma	21740	38	0.24	1	69	0.10
SCL39	Serum	Autoimmune	Scleroderma	300700	18	3.33	146	293	5.58
SCL40	Serum	Autoimmune	Scleroderma	406916	34	4.50	139	280	5.78

1.4 Specificity

Specificity relates to the ability of the test to identify negative results. It is the statistical probability that an individual who does not have the particular disease being tested for will be correctly identified as negative. The specificity of this Anti Scl-70 assay, towards samples containing antibodies specific for other ANA-related disorders, was tested on Theranos systems. Five RF positives, five HAMA positives, and positive controls for 11 ANA-related disorders from Centers For Disease Control (CDC) were tested with this Anti Scl-70 assay on Theranos system. Of the 21 samples tested, only the CDC anti-Sm and anti-Scl-70 controls tested positive for this assay (data is summarized in Table 3). Ideally, none of the samples should test positive with the exception of the anti Scl-70 control. For this reason, additional screening with Biorad's Anti-Sm positive control was performed for confirmation, but result tested negative. Biorad Anti-RNP positive control was also tested because RNP sometimes gives positive reading for samples containing Anti-Sm antibodies. However, result for the Anti-RNP control was also negative. Additional experiment was therefore conducted to exclude the probability of reactivity occurring from using the CDC Anti-Sm control by spiking Scl-70 antigen directly into the CDC Anti-Sm control to block the formation of Scl-70 antibody. However, this experiment yielded positive result, thus suggesting that the reactivity must be due to an excipient(s) within the CDC anti-Sm control sample matrix. To exclude the notion of false positives occurring due to the possible existence of heterophilic antibodies and their potential for causing interference in this assay, additional test was performed with heterophilic blocking reagent (HBR). Even with HBR, the CDC Anti-Sm control remained positive, thus indicating that the positive test result was unrelated to heterophilic antibodies interferences. Finally, to further demonstrate that this Anti Scl-70 assay is not specific for Anti-Sm antibodies, four clinical sera that tested positive on the Anti-Sm assay were screened on the Theranos system using conditions finalized for the Anti Scl-70 assay. Data from the analysis showed that all four samples tested negative under anti Scl-70 assay conditions. Based on this data, at least two conclusions can be made: (1) the positive test result of the CDC anti-Sm control is a false positive caused by an excipient(s) in its matrix that is unrelated to Anti Scl-70 antibodies, and (2) clinical samples that are specific for Anti-Sm antibodies will not test positive on this Anti Scl-70 assay. Therefore, this assay is considered specific only for Anti Scl-70 antibodies. The data generated as a result of troubleshooting "Specificity" are summarized in Table 4. Demographics information pertaining to the clinical samples tested are available in Table 5.

Table [SEQ Table * ARABIC]. Specificity Data

Sample Info	Ab Index	INOVA Ab Index
CDC#1 Positive ANA (Homog/Rim) & Positive Anti-Native DNA	0.86	N/A
CDC#2 Positive ANA (speckled) & Positive Anti-SS-B	0.15	
CDC#3 Positive ANA (speckled)	0.21	
CDC#4 Positive Anti-RNP	0.61	
CDC#5 Positive Anti-Sm	2.99	
CDC#6 Positive ANA (nucleolar)	0.33	
CDC#7 Positive ANA SSA/Ro	0.30	
CDC#8 Positive ANA (centromere)	0.13	
CDC#9 Positive Anti Scl-70	8.56	
CDC#10 Positive Anti Jo-1	0.48	
CDC#12 Positive Anti-Ribosomal P	0.61	
HAMA positive #5	0.35	5
HAMA positive #7	0.66	2
HAMA positive #15	0.43	6
HAMA positive #19	0.26	1
HAMA positive #24	0.26	1
RF positive #19	0.33	1
RF positive #20	0.34	1
RF positive #23	0.18	1
RF positive #24	0.17	1
RF positive #25	0.29	5

Table [SEQ Table * ARABIC]. Specificity Troubleshoot Data

Sample ID	Matrix	Inter-Cartridge		ANTIBODY INDEX
		Mean	%CV	Theranos
Biorad Anti-Sm Positive Control	Serum	1928	15	0.02
Biorad Anti-RNP Positive Control	Serum	2203	15	0.02
CDC Anti-Sm Positive Control (Neat)	Serum	206909	12	2.29
CDC Anti-Sm Positive Control (1:2)	Serum	235858	7	2.61
CDC Anti-Sm Positive Control (1:10)	Serum	82713	9	0.92
CDC Anti-Sm (spiked with 2.5 µg/mL Scl-70 Ag)	Serum	241091	6	2.67
CDC Anti-Sm (spiked with 400 µg/mL HBR)	Serum	317624	12	3.52
Positive Clinical Sample for Anti-Sm Assay (SLE4)	Serum	16477	15	0.18
Positive Clinical Sample for Anti-Sm Assay (SLE7)	Serum	15123	40	0.17
Positive Clinical Sample for Anti-Sm Assay (SLE9)	Serum	15454	22	0.17
Strong Positive Clinical Sample for Anti-Sm Assay (SCL14)	Serum	69211	19	0.77

Table [SEQ Table * ARABIC]. Clinical Demographics Data

Human Clinical Samples				Gender	Age
Sample ID	Matrix	Species	Strain		
CLS1	Serum	Autoimmune	Scleroderma	Female	76
CLS2	Serum	Autoimmune	Scleroderma	Female	44
CLS9	Serum	Autoimmune	Scleroderma	Female	32
SS10	Serum	Autoimmune	Sjogren	Female	35
SCL06	Serum	Autoimmune	Scleroderma	Female	44
SCL16	Serum	Autoimmune	Scleroderma	Female	78
SCL25	Serum	Autoimmune	Scleroderma	Female	62
SCL29	Serum	Autoimmune	Scleroderma	Female	28
SCL38	Serum	Autoimmune	Scleroderma	Female	74
SCL39	Serum	Autoimmune	Scleroderma	Female	55
SCL40	Serum	Autoimmune	Scleroderma	Female	51
H5	Serum	Interference Serum	HAMA	Male	26
H7	Serum	Interference Serum	HAMA	Male	51
H15	Serum	Interference Serum	HAMA	Male	54
H19	Serum	Interference Serum	HAMA	Male	42
H24	Serum	Interference Serum	HAMA	Male	18
R19	Serum	Autoimmune	RF	Female	70
R20	Serum	Autoimmune	RF	Female	60
R23	Serum	Autoimmune	RF	Female	73
R24	Serum	Autoimmune	RF	Male	59
R25	Serum	Autoimmune	RF	Male	59

1.5 Stability

- Stability testing of detection antibody conjugate and surface coated with capture antibody is ongoing.

2 CONCLUSION

We have successfully developed an immunoassay to detect Anti-Scl70 in human serum and plasma.