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Anti – RNP
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"]

RNP antigen is a 68 kilo-Dalton (kD) ribonucleoprotein. Anti-RNP antibodies react with proteins that are associated with U1 RNA and form U1snRNP. There is a high incidence of RNP autoantibody in patients with collagen diseases such as systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD). MCTD (also known as Sharp's syndrome) is an autoimmune disease that is considered as an overlap of three diseases: SLE, scleroderma and polymyositis. SLE is a Type III hypersensitivity reaction caused by antibody-immune complex formation. It most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called flares) alternating with remissions. SLE occurs nine times more often in women than in men, especially in women in child-bearing years ages 15 to 35. Anti-RNP antibodies are detectable in 25-47% of SLE patients, most notably those of African descent. When present alone at high levels in blood, Anti-RNP antibodies are diagnostic of MCTD (with an incidence reportedly as high as 98.5%). Lower levels of anti-RNP, in conjunction with other autoantibodies, may be observed in scleroderma, Sjogren's Syndrome and Rheumatoid Arthritis. Anti-RNP antibodies are also more prevalent in patients with Raynaud's phenomenon and are associated with milder renal involvement.

This assay is designed to qualitatively determine anti-RNP antibodies (Ab) 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® RNP ELISA (Cat# 708565)
- Corgenix REAADS Anti-RNP ELISA (Cat# 10869)*
- IBL U1-RNP IgG ELISA (Cat# RE75211)

**FDA-cleared*

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

An RNP antigen coated surface serves as the capture surface for the RNP assay. The sample (plasma or serum) is diluted and incubated on the capture surface for 10 minutes. The surface is then washed to remove unbound proteins. An alkaline phosphatase (AP)-labeled anti-human IgG antibody is then incubated on the surface for 10 minutes. After the detection antibody incubation, another washing cycle is performed and the alkaline phosphatase substrate is incubated on the surface for 10 minutes, and the resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #	Lot #
Antigen: RNP-68k antigen native	Arotec	ATR04-02	K9011503
Clone 1, Mouse Anti-Human IgG1 Antibody, 2C11	Novus Biologicals	NB100-2046	10/12-G2-C11
Clone 2, Mouse Anti-Human IgG Antibody, JDC10	Southern Biotech	9040-01	L0810-SD31
Clone 3, Goat F(ab')2 Anti-Human IgG Antibody	Southern Biotech	2042-01	C5711-SG21
Biotin-SH Labeling Kit	Dojindo	LK10	ES613
Alkaline Phosphatase Labeling Kit	Dojindo	LK13-10	ES614
PhosphoGlo Substrate (Commercial)	KPL	55-60-04	120380, 120127
Theranos Substrate	In-House	N/A	T-ALKP-SB01-001, T-ALKP-SB01-004
Carbonate Bicarbonate Buffer (CBC)	In-House	N/A	NB362-CL-25A
Blocking Buffer (BB) (3% BSA in TBS, 0.05% Sodium Azide)	In-House	N/A	NB362-CL-39A
Wash Buffer	In-House	N/A	362-CL-123B
Theranos AP Antibody Conjugate Stabilizer	In-House	N/A	NB408-CL-45A
Biostab Stabilizer	Fluka	76696	BCBB3963
StabilZyme Stabilizer	Surmodics	SA01-1000	SA01L18
SuperBlock® Blocking Buffer in TBS	Thermo	37535	NC168883
StartingBlock™ Blocking Buffer in TBS	Thermo	37542	ND169707
Blocking Buffer + 400 µg/mL HBR	In-House	N/A	NB408-CL-54A
Normal Serum (CLN1 through CLN15)	Stanford Blood Bank Center	N/A	N/A
Scleroderma Clinical Sera (CLS1 – CLS10; SCL01 – SCL42)	Bioreclamation	N/A	N/A
Sjogren Clinical Sera (SS1 – SS10)	Bioreclamation	N/A	N/A
Systemic Lupus Erythematosus Clinical Sera (SL01 – SL10) & (CSLE1 – CSLE15)	Bioreclamation	N/A	N/A
Mixed Connective Tissue Disease Clinical Sera (MCTD1 – MCTD5)	Bioreclamation	N/A	N/A
Human-Anti-Mouse Antibody (HAMA) Positive Sera (#H8,H14,H16,H17,H18), MMRV Panel	ProMedDx	N/A	N/A
Rheumatoid Factor (RF) Positive Sera (R21,R22,R24,R25,R12), MMRV Panel	ProMedDex	N/A	N/A
Liquicheck Anti-RNP Positive Control	BioRad	116	20710
Liquicheck Autoimmune Negative Control	BioRad	130	17440

Name	Supplier	Catalog #	Lot #
ANA Human Reference Serum #1 (Pos ANA (Homog/Rim) & Pos Anti-Native DNA)	U.S National Reference Serum (CDC)	IS2072	98-0026L
ANA Human Reference Serum #2 (Pos ANA (Speckled) & Pos Anti-SS-B)	U.S National Reference Serum (CDC)	IS073	82-0008
ANA Human Reference Serum #3 (Pos ANA (Speckled))	U.S National Reference Serum (CDC)	IS074	82-0009
ANA Human Reference Serum #4 (Pos Anti-RNP)	U.S National Reference Serum (CDC)	IS075	95-0055L
ANA Human Reference Serum #5 (Pos Anti-Sm)	U.S National Reference Serum (CDC)	IS2076	96-0005L
ANA Human Reference Serum #6 (Pos ANA (nucleolar))	U.S National Reference Serum (CDC)	IS2100	82-0141
ANA Human Reference Serum #7 (Pos ANA (SSA/Ro)	U.S National Reference Serum (CDC)	IS2105	83-0026
ANA Human Reference Serum #8 (Pos ANA (Centromere))	U.S National Reference Serum (CDC)	IS2134	84-0026
ANA Human Reference Serum #9 (Pos Anti-Scl70)	U.S National Reference Serum (CDC)	IS2135	84-0027
ANA Human Reference Serum #10 (Pos Anti J0-1)	U.S National Reference Serum (CDC)	IS2197	88-024
ANA Human Reference Serum #12 (Pos Anti-Ribosomal P)	U.S National Reference Serum (CDC)	IS2706	04-0169

2 ASSAY DEVELOPMENT[TC "ASSAY OPTIMIZATION" \F C \L "2"]

2.1 Capture Surface Screen [TC "Capture Surface screen" \f C \l "1"]

The best capture surface for the anti-RNP assay was initially intended to be evaluated by comparing unlabeled RNP antigen capture surface to that of biotin-labeled RNP antigen surface. However, the RNP antigen (Arotec, CAT# ATR-0402) proved difficult to label via biotin conjugation. There was no protein recovery. Therefore, it was decided that capture surface would be best prepared with unlabeled RNP antigen through direct coating.

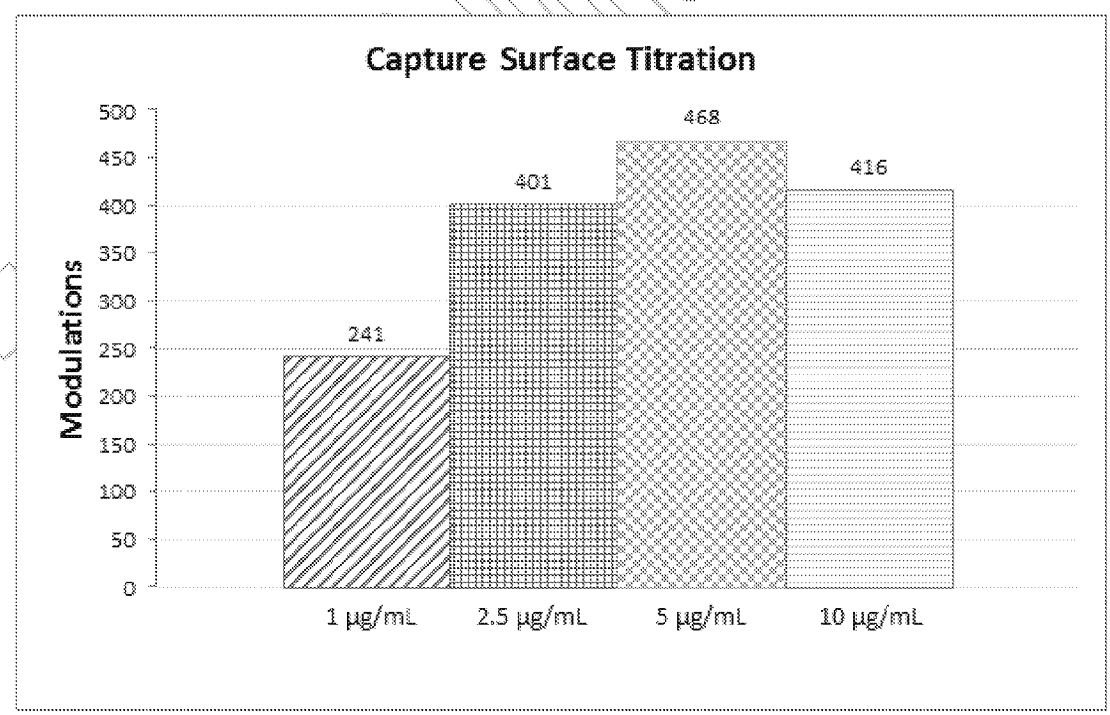
2.1 Capture Surface Titration[TC " Capture Surface Titration " \f C \l "1"]

Unlabeled RNP antigen in CBC was titrated at four levels: 1, 2.5, 5 and 10 µg/mL and tested against Biorad positive and negative controls, 1 pooled normal serum sample (tested negative for RNP antibody via all three predicate methods listed in section 1.1.1) and 1 clinical serum sample (tested positive for RNP antibody via all 3 predicate methods). The coating condition at 5 µg/mL provided the highest modulation between the RNP positive clinical sample and the Biorad negative control, and therefore was selected as the final condition for the capture surface. Data is summarized in Table 2.

Table [SEQ Table * ARABIC]. Capture Surface Titration

Samples	1 µg/mL		2.5 µg/mL		5 µg/mL		10 µg/mL	
	Inter-Cartridge Data							
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	28855	15	99666	13	242543	12	534314	1
Biorad Negative Control	317	4	457	21	1105	9	1783	9
RNP Positive Clinical	76260	17	182952	23	516634	10	741387	2
Stanford Normal	516	43	627	2	991	6	1752	4
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	241		401		468		416	
S/B Modulation (Biorad Neg Control & Biorad RNP Pos Ctrl)	91		218		220		300	

Figure [SEQ Figure * ARABIC]. Capture Surface Titration



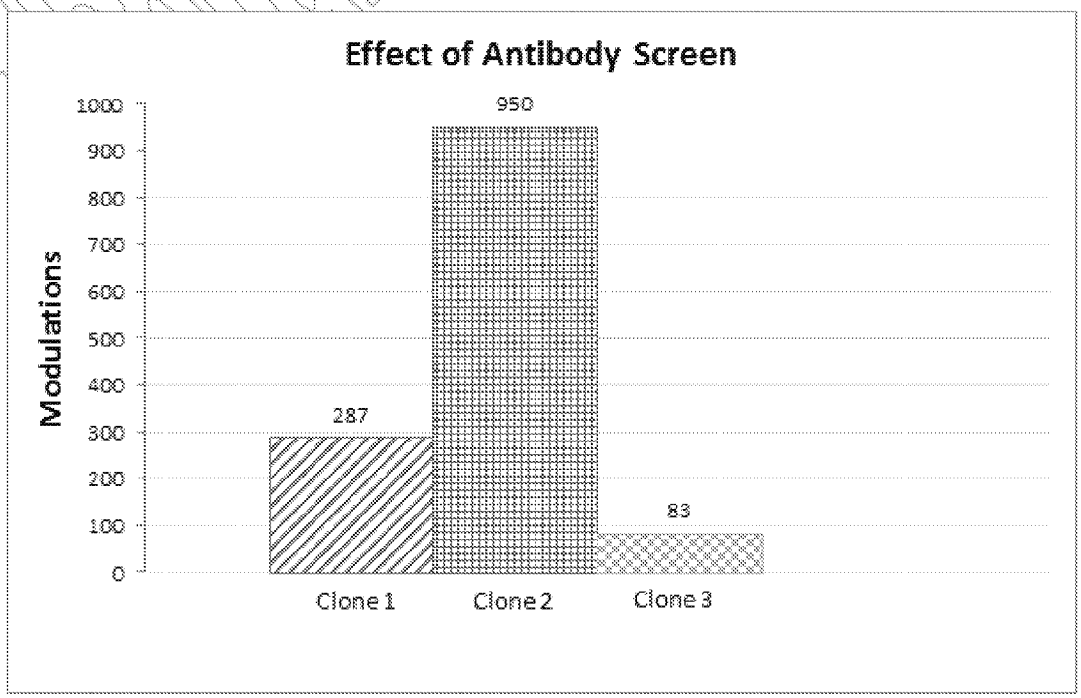
2.2 Detection Antibody Screen

Detection antibody screening was performed with three clones of anti-human IgG: Clone 1 (Novus Biologicals), Clone 2 (Southern Biotech) and Clone 3 (Southern Biotech). All three clones were conjugated to alkaline phosphatase (AP). The clone 2 provided the highest modulation between the commercial negative control and the RNP positive clinical serum. However, its background signal is excessively high as observed by both the Biorad negative control and Stanford normal. Therefore, clone 1, which afforded the next best modulation, was deemed the better option. Data is shown below in Table 3.

Table [SEQ Table * ARABIC]. Detection Antibody Screen

Control	Clone 1		Clone 2		Clone 3	
	Inter-Cartridge RLU					
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	144721	29	1462941	3	2729663	9
Biorad Negative Control	1123	19	1684	5	24511	26
RNP Positive Clinical	321959	17	1600112	10	2043347	7
Stanford Normal	925	21	11512	46	60200	14
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	287		950		83	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	129		869		111	

Figure [SEQ Figure * ARABIC]. Detection Antibody Screen



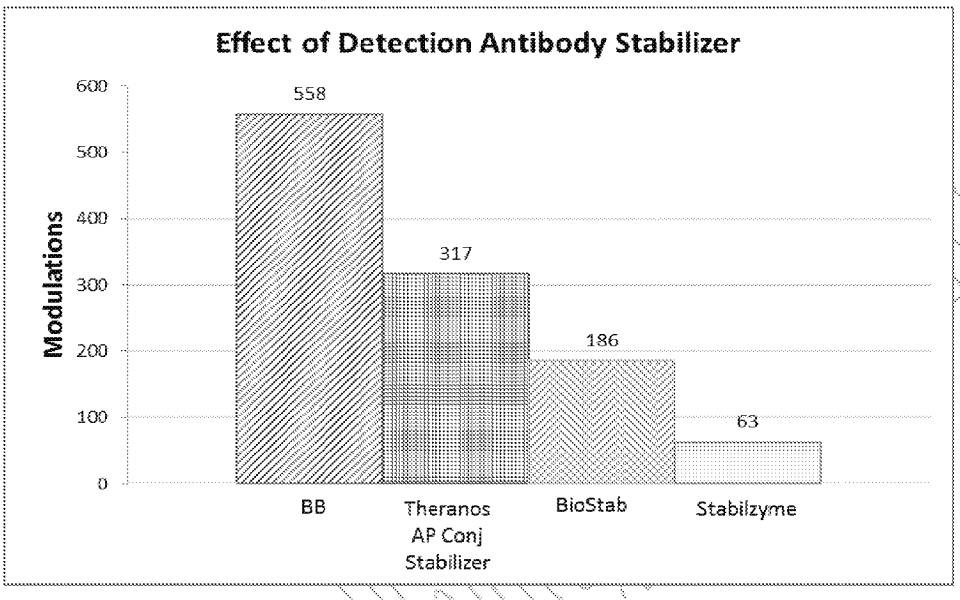
2.3 Effect of Alkaline Phosphatase Conjugate Stabilizer[TC "Effect of alkaline phosphatase conjugate stabilizer " \f C \l "1"]

Two commercial stabilizers (Biostab and StabilZyme) and one in house formulated alkaline phosphatase (AP) stabilizer were tested against the 3% BSA blocking buffer in TBS as detection antibody diluents, with the anti-human detection antibody (Dab) prepared at a final working concentration of 100 ng/mL. The Theranos-formulated AP stabilizer consisted of 3% BSA blocking buffer in TBS spiked with $ZnCl_2$ and $MgCl_2$ to achieve a final concentration of 0.1 mM Zn^{2+} and 5 mM Mg^{2+}. The samples were diluted 1:25 into 3% BSA blocking buffer in TBS. Signal modulation was observed to be highest with both Blocking Buffer and Theranos AP Conjugate Stabilizer. However, for long term stability and storage, the latter proved to be a better choice. Therefore, Theranos AP conjugate stabilizer was finalized as the AP conjugate stabilizer for this assay. Data is captured in Table 4.

Table [SEQ Table * ARABIC]. Effect of AP Conjugate Stabilizers

Samples	Blocking Buffer		Theranos AP Conjugate Stabilizer		BioStab		StabilZyme	
	Inter-Cartridge Data							
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	180126	32	280956	10	110704	39	43294	21
Biorad Negative Control	585	5	1007	8	1018	38	1208	21
RNP Positive Clinical	326543	7	400017	33	314207	42	76385	28
Stanford Normal	1200	24	1929	24	1678	40	932	109
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	558		317		186		63	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	336		279		83		36	

Figure [SEQ Figure * ARABIC]: Effect of Alkaline Phosphatase Conjugate Stabilizer



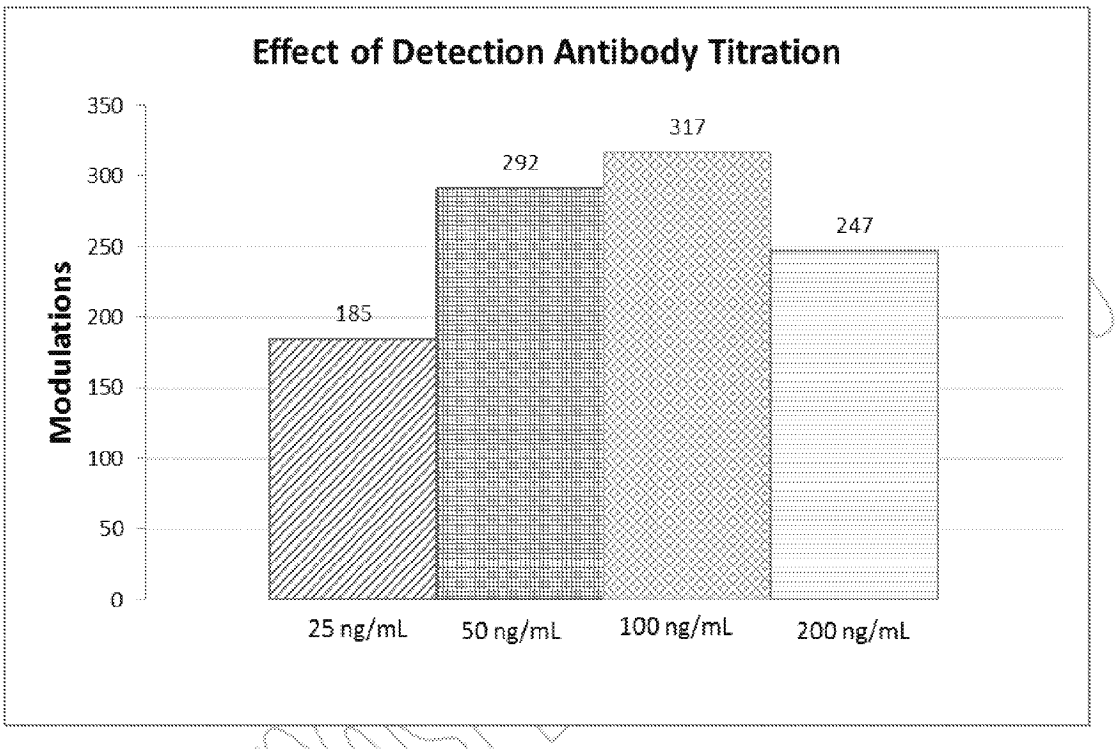
2.4 Detection Antibody Titration

The AP conjugated detection antibody was evaluated in Biostab at four concentration levels: 25 ng/mL, 50 ng/mL, 100 ng/mL and 200 ng/mL. As suggested by the results shown in Table 5, the best modulation between the positive and negative controls was achieved with 100 ng/mL of the anti-human IgG detection antibody. It was therefore finalized as the final concentration of the detection antibody conjugate. Data is summarized in Table 5.

Table [SEQ Table * ARABIC]. Detection Conjugate Titration

Samples	25 ng/mL		50 ng/mL		100 ng/mL		200 ng/mL	
	Inter-Cartridge Data							
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	59897	17	117460	0	280956	10	487806	15
Biorad Negative Control	455	30	598	30	1007	8	2134	14
RNP Positive Clinical	84214	2	174909	10	319374	24	526217	17
Stanford Normal	470	4	606	19	1578	10	2136	39
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	185		292		317		247	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	132		196		279		229	

Figure [SEQ Figure * ARABIC]: Detection Titration



2.5 Effect of Assay Diluent

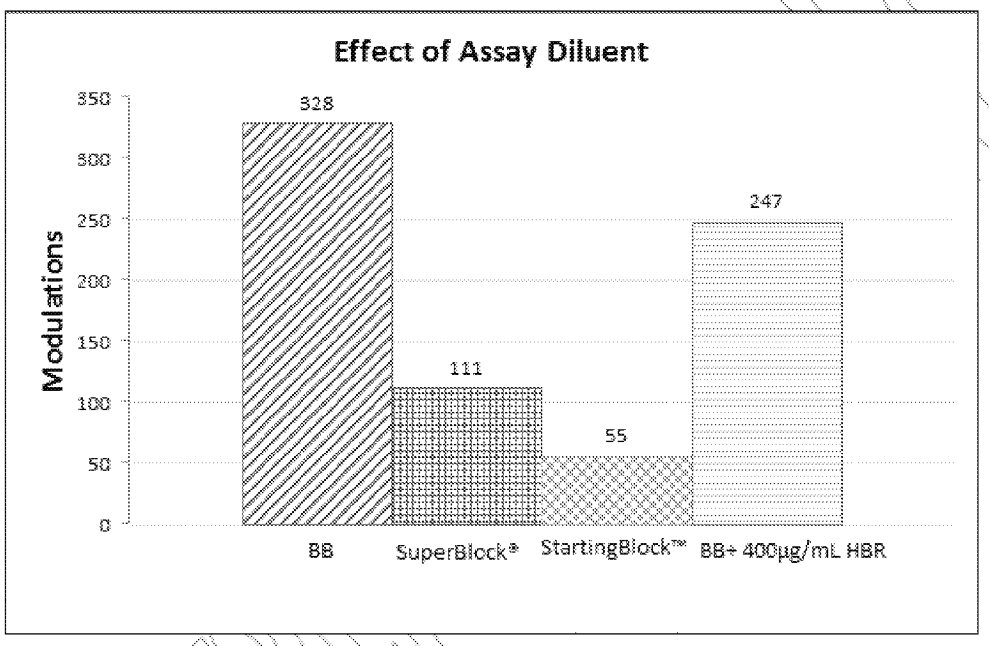
Two commercially available blockers (SuperBlock® and StartingBlock™) and one in-house blocking buffer spiked with 400 µg/mL of HBR were tested as diluents for the assay. Data was compared to the control diluent which was the blocking buffer consisted of 3% BSA and 0.05% sodium azide in TBS. The control condition produced the best modulation out of all the diluents tested with respect to the Biorad positive and negative controls. 3% BSA blocking buffer in TBS (without HBR) was therefore finalized as the diluent of choice for this assay. Refer to data in Table 6.

Table [SEQ Table * ARABIC]. Effect of Assay Diluent

Sample	Control: Blocking Buffer		SuperBlock®		StartingBlock™		Blocking Buffer + 400 µg/mL HBR	
	Inter-Cartridge Data							
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	215637	15	57173	1	34886	38	215630	15
Biorad Negative Control	657	15	516	26	640	18	873	5
RNP Positive Clinical	2712	8	3267	2	1540	19	2638	7
Stanford Normal	930	16	1060	17	1015	35	1152	7
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	4		6		2		3	

S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	328	111	55	247

Figure [SEQ Figure * ARABIC]. Effect of Assay Diluent



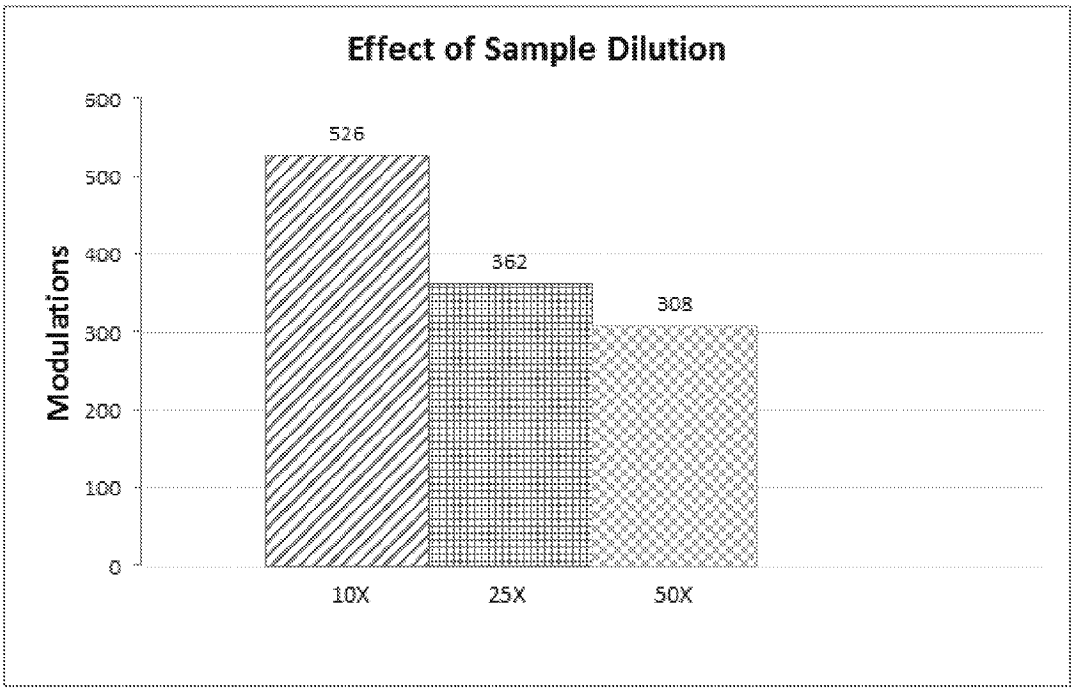
2.6 Effect of Sample Dilution

The effect of sample dilution was tested with final sample dilution factors of 1:10, 1:25 and 1:50 in blocking buffer consisted of 3% BSA and 0.05% Sodium Azide in TBS. Modulation between positive and pooled negative sera was greatest at 1:10. However, 25X also provided excellent modulation. 25X is preferred because higher dilution allows for more samples to be available in the event this becomes a part of a multiplex assay. Therefore, 1:25 dilution was chosen as the sample dilution for this anti-RNP assay. Results are summarized in Table 7.

Table [SEQ Table * ARABIC]. Effect of Sample Dilution

Samples	10X		25X		50X	
	Inter-Cartridge Data					
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	350245	6	245310	38	131144	30
Biorad Negative Control	859	31	863	19	861	16
RNP Positive Clinical	451487	12	311931	9	265192	14
Stanford Normal	1392	19	1129	13	1218	25
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	526		362		308	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	408		284		152	

Figure [SEQ Figure * ARABIC]: Effect of Sample Dilution



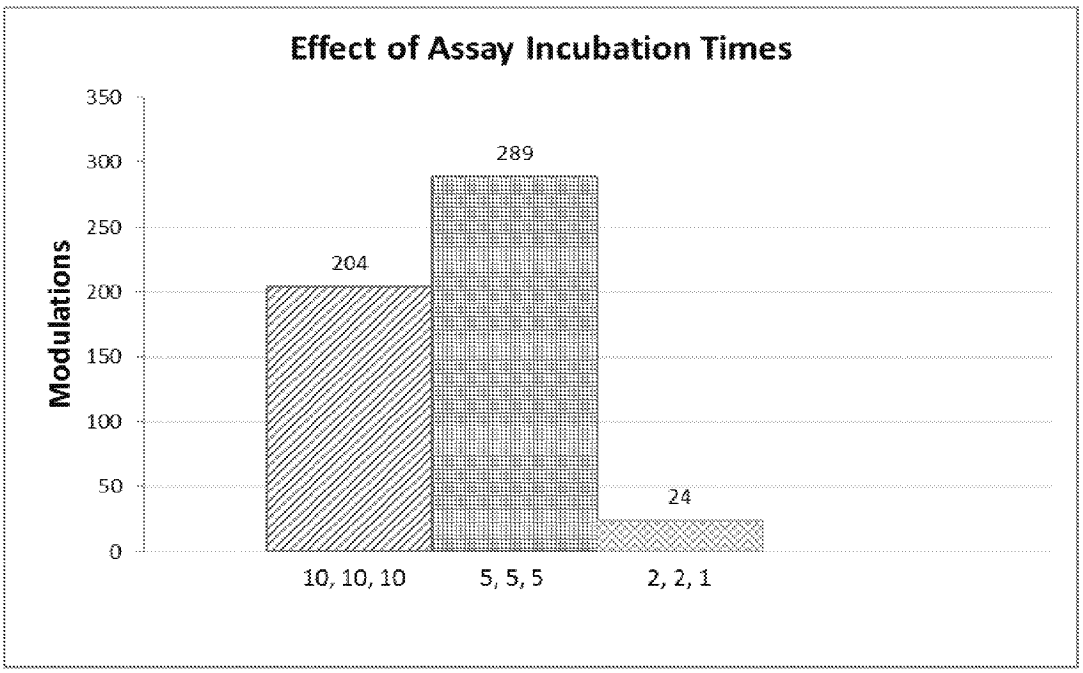
2.7 Effect of Incubation Time

The effect of shorter time to incubate sample, detection conjugate and substrate was evaluated at 5, 5, 5 and 2, 2, 1 minutes for comparison against the 10, 10, 10 minutes incubation time. Assay modulation between the RNP positive clinical sample and the Biorad negative control was almost similar between the 10, 10, 10 and 5, 5, 5 incubation times. However, 10, 10, 10 incubation time offered better modulation between Biorad controls. Therefore, it was selected as the final condition.

Table [SEQ Table * ARABIC]. Effect of Reagent Incubation Time

Samples	10, 10, 10 min		5, 5, 5 min		2, 2, 1 min	
	Inter-Cartridge Data					
	Mean RLU	%CV	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	269562	0	48714	21	6449	22
Biorad Negative Control	1698	5	400	16	624	23
RNP Positive Clinical	345956	7	115772	16	15233	6
Stanford Normal	2428	8	1045	9	760	9
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	204		289		24	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	159		122		10	

Figure [SEQ Figure * ARABIC]: Effect of Incubation Time



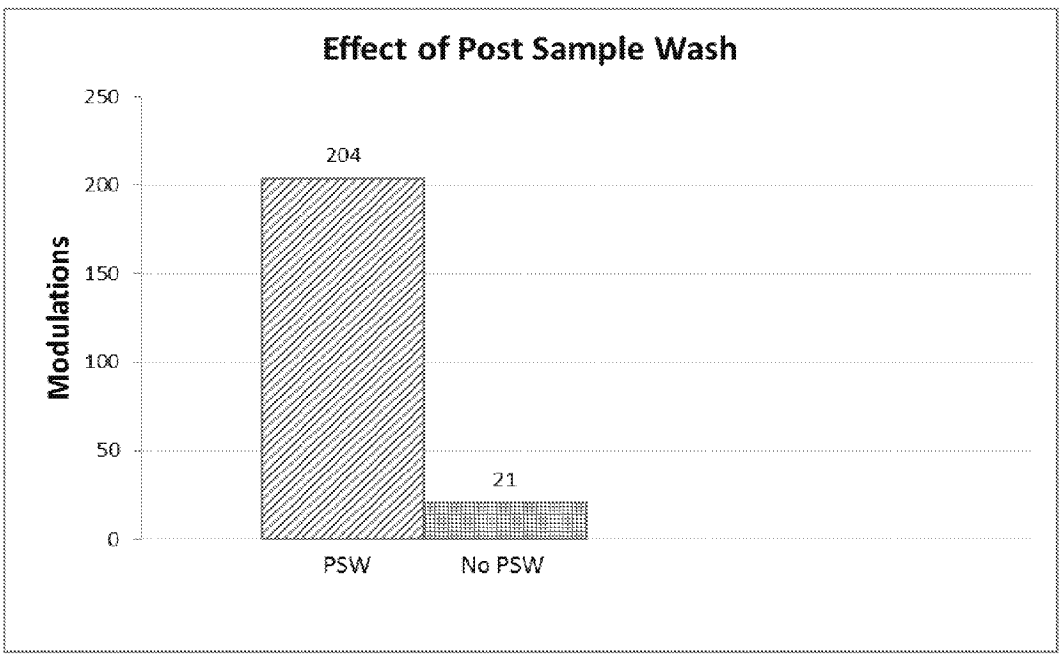
2.8 Effect of Post Sample Wash

The effect of a post sample wash was tested with the capture surface coated at 5 µg/mL (with Arotec RNP/Sm-free antigen), using a 1:25 sample dilution and detection antibody prepared at 100 ng/mL in Theranos AP Conjugate Stabilizer. The assay performed most optimally with post sample wash since this provided the best modulation between the commercial negative control and positive samples and afforded better precision. Data is shown in Table 9.

Table [SEQ Table * ARABIC]. Effect of Post Sample Wash

Sample	25X_PSW		25X (No PSW)	
	Inter-Cartridge Data			
	Mean RLU	%CV	Mean RLU	%CV
Biorad Positive Control	269562	0	306570	9
Biorad Negative Control	1698	5	5746	58
RNP Positive Clinical	345956	7	121054	77
Stanford Normal	2428	8	4330	22
S/B Modulation (Biorad Neg Control & RNP Pos Clinical)	204		21	
S/B Modulation (Biorad Neg Control & Biorad Pos Ctrl)	159		53	

Figure [SEQ Figure * ARABIC]: Effect of Post Sample Wash



2.9 Normal Sample Screen: Cut-off Determination

Twenty-seven (27) randomly selected normal donor serum samples obtained from Stanford blood bank center and Bioreclamation were screened on the Theranos system to determine the cut-off value, which was calculated to be 181,078 RLU. The Theranos cutoff value was determined by taking the mean RLU of the 27 normal samples plus 5 times the standard deviation. The sample RLU divided by the cutoff value yields its Antibody Index. Samples are considered to be positive, borderline, or negative for RNP 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 27 samples were also screened using the predicate methods listed in section 1.1.1. There was excellent correlation between Theranos results and those obtained from the predicate methods since all 26 samples tested negative across all platforms with one borderline. Table | SEQ Table * ARABIC |. Normal Donor Samples Screen on Theranos vs Corgenix, INOVA & IBL RNP ELISA

Sample ID	Source	Matrix	Inter-Cartridge		ANTIBODY INDEX			
			Mean	%CV	Theranos	Corgenix	INOVA	IBL
CLN1	Stanford	Serum	1270	6	0.01	3.28	4	0.19
CLN2	Stanford	Serum	1397	5	0.01	1.64	4	0.08
CLN3	Stanford	Serum	2330	8	0.01	3.31	6	0.27
CLN4	Stanford	Serum	1641	22	0.01	2.77	5	0.08
CLN5	Stanford	Serum	1616	7	0.01	2.89	4	0.06
CLN6	Stanford	Serum	2130	34	0.01	2.58	7	0.16

Note: Table 10 continues on next page.

Table 10 (continued): Normal Donor Samples Screen on Theranos vs Corgenix, INOVA & IBL Scl-70 ELISA

Sample ID	Source	Matrix	Inter-Cartridge		ANTIBODY INDEX			
			Mean	%CV	Theranos	Corgenix	INOVA	IBL
CLN7	Stanford	Serum	1596	14	0.01	3.33	6	0.19
CLN8	Stanford	Serum	3574	12	0.02	5.67	5	0.19
CLN9	Stanford	Serum	178971	29	0.99	1.69	4	0.12
CLN10	Stanford	Serum	13087	12	0.07	3.32	5	0.12
CLN11	Stanford	Serum	12703	5	0.07	2.44	4	0.15
CLN12	Stanford	Serum	14732	5	0.08	6.66	10	0.20
CLN13	Stanford	Serum	7627	13	0.04	1.98	5	0.09
CLN14	Stanford	Serum	5716	17	0.03	1.89	4	0.08
CLN15	Stanford	Serum	2378	17	0.01	4.21	4	0.10

Note: Table 10 continues on next page.

Table 10 (continued): Normal Donor Samples Screen on Theranos vs Corgenix, INOVA & IBL Scl-70 ELISA

Sample ID	Source	Matrix	Inter-Cartridge		ANTIBODY INDEX			
			Mean	%CV	Theranos	Corgenix	INOVA	IBL
MCTD1	Bioreclamation	Serum	2353	16	0.01	1.40	6	0.29
MCTD2	Bioreclamation	Serum	2729	24	0.02	1.58	8	0.09
MCTD3	Bioreclamation	Serum	3449	4	0.02	1.72	6	0.14
MCTD4	Bioreclamation	Serum	1111	17	0.01	3.18	6	0.05
MCTD5	Bioreclamation	Serum	2225	13	0.01	2.30	6	0.11
CSLE1	Bioreclamation	Serum	1919	23	0.01	1.20	7	0.14
CSLE3	Bioreclamation	Serum	1771	48	0.01	0.51	5	0.13
CSLE6	Bioreclamation	Serum	1801	17	0.01	2.35	7	0.16
CSLE9	Bioreclamation	Serum	7658	15	0.03	4.50	6	0.13
CSLE12	Bioreclamation	Serum	1558	18	0.01	1.19	7	0.10
SCL01	Bioreclamation	Serum	16801	17	0.09	4.09	7	0.14
SCL03	Bioreclamation	Serum	20858	45	0.12	2.42	6	0.16
Overall MEAN			11576					
Overall STDEV			33900					
CUT OFF			181078					

2.10 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-RNP 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. Of the 21 samples tested, four CDC controls (ANA Speckled, Anti-RNP, Anti-Sm and Anti-SSA) tested positive for this assay (data is summarized in Table 11). Ideally, none of the samples should test positive with the exception of the anti-RNP control. However, it is expected that Anti-ANA Speckled and Anti-Sm should test positive for this Anti-RNP assay because both of these CDC controls contain RNP. The Anti-SSA control however did not contain RNP but yet was tested positive on the Theranos systems. For this reason, additional screening with Biorad's Anti-SSA positive control was performed for confirmation, but result tested negative. To further demonstrate that this Anti RNP assay is not specific for Anti-SSA antibodies, one clinical serum that tested as a strong positive on the Anti-SSA assay was screened on the Theranos system using conditions finalized for the Anti-RNP assay. Data from the analysis for this clinical sample yielded very low titer for Anti-RNP antibodies which resulted in a negative reading under the Anti-RNP assay conditions. Based on this data, at least two conclusions can be made: (1) the positive test result of the CDC anti-SSA control is a false positive caused by an excipient(s) in its matrix that is unrelated to Anti-RNP antibodies, and (2) clinical sample that is specific for Anti-SSA antibodies will not test positive on this Anti-RNP assay. Therefore, this assay is considered specific only for Anti-RNP antibodies. The data generated as a result of troubleshooting "Specificity" are summarized in Table 12. Demographics information pertaining to the clinical samples tested are available in Table 14.

Table [SEQ Table * ARABIC]. Specificity Data

Sample Info	Theranos Ab Index	Corgenix Ab Index
CDC#1 Positive ANA (Homog/Rim) & Positive Anti-Native DNA	0.19	8.25
CDC#2 Positive ANA (speckled) & Positive Anti-SS-B	0.03	1.97
CDC#3 Positive ANA (speckled)	3.92	173.45
CDC#4 Positive Anti-RNP	4.11	147.79
CDC#5 Positive Anti-Sm	1.69	N/A
CDC#6 Positive ANA (nucleolar)	0.07	2.20
CDC#7 Positive ANA SSA/Ro	2.33	0.48
CDC#8 Positive ANA (centromere)	0.01	0.69
CDC#9 Positive Anti Scl-70	0.10	3.42
CDC#10 Positive Anti Jo-1	0.04	0.23
CDC#12 Positive Anti-Ribosomal P	0.03	0.53
HAMA positive #8	0.16	5
HAMA positive #14	0.16	2
HAMA positive #16	0.21	6
HAMA positive #17	0.32	1
HAMA positive #18	0.17	1
RF positive #21	0.13	1
RF positive #22	0.03	1
RF positive #24	0.03	1
RF positive #25	0.07	1
RF positive #12	0.37	5

Table [SEQ Table * ARABIC]. Specificity Troubleshoot Data

Sample ID	Matrix	Inter-Cartridge		ANTIBODY INDEX
		Mean	%CV	Theranos
Biorad Anti-SSA Positive Control	Serum	1137	7	0.01
Strong Positive Clinical Sample for Anti-SSA Assay (SLE1)	Serum	2605	13	0.01

2.11 Clinical Sample Correlation

Clinical correlation assesses the accuracy of this anti-RNP assay with respect to the predicate methods listed in section 1.1.1. Randomly obtained normal and clinical serum samples 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 RNP antibodies.

The commercial ELISA kits were obtained from three different vendors: INOVA Diagnostics, Corgenix and IBL International. 107 normal and clinical sera obtained from Stanford and Bioreclamation were collectively screened on these kits. INOVA, Corgenix and IBL each yielded 18, 17, and 14 positives, respectively, out of all the samples screened. Of the 107 samples, 20 of those samples screened positive on at least one of the three commercial kits (while only 13 of those samples tested positive on all 3 kits). All 20 samples that screened positive on any of the three kits (in addition to the 10 out of 15 normal Stanford sera) were screened on the Theranos system to evaluate clinical correlation. 8 out of the 18 clinical sera that tested positive for RNP antibody on the INOVA Scl-70 ELISA kit also tested positive on the Theranos system. One additional serum that tested positive on both the IBL and Corgenix RNP kits (but not on the INOVA kit) was also screened on the Theranos system. It was found to be negative, in correlation with the INOVA kit. Out of all the normal sera that tested negative across all three commercial ELISA kits, all also tested negative on the Theranos system. There was an approximately 62% correlation among the Theranos Anti-RNP assay and the 3 commercial ELISA kits data. This was calculated by taking the percentage of the number of positives yielded by the Theranos system (n=8) versus the number of positives agreed upon by all 3 predicate methods (n=13). However, only a small population of normal samples (27 total) was evaluated for cutoff (refer to section 2.9). Correlation between Theranos Anti-RNP ELISA and the predicate methods could be improved by screening more normal samples and re-calculating the cutoff based on a larger pool of normals. This will be further demonstrated during validation. See Table 10 for data from the normal donor sample screen on Theranos system in relation to the predicate methods. See Table 13 for a comparison of clinical correlation data between Theranos system and the predicate methods. Note that the normal Stanford sera (CLN1 to CLN10) were evaluated twice on the Theranos system on different days to provide data for: (1) normal screening (to determine cutoff) and (2) clinical correlation. Hence, Tables 10 and 13 contain different Theranos data sets for samples CLN1 to CLN10.

Table [SEQ Table * ARABIC]. Clinical Correlation Data

Sample ID	Human Test Samples			Inter-Cartridge		ANTIBODY INDEX			
	Matrix	Species	Strain	Mean	%CV	Theranos	INOVA	Corgenix	IBL
CLN1	Serum	Normal	N/A	1410	12	0.01	4	3.28	0.19
CLN2	Serum	Normal	N/A	1319	17	0.01	4	1.64	0.08
CLN3	Serum	Normal	N/A	1863	25	0.01	6	3.31	0.27
CLN4	Serum	Normal	N/A	1475	24	0.01	5	2.77	0.08
CLN5	Serum	Normal	N/A	1486	26	0.01	4	2.89	0.06
CLN6	Serum	Normal	N/A	2449	27	0.01	7	2.58	0.16
CLN7	Serum	Normal	N/A	1242	9	0.01	6	3.33	0.19
CLN8	Serum	Normal	N/A	2350	17	0.01	5	5.67	0.19
CLN9	Serum	Normal	N/A	1322	18	0.01	4	1.69	0.12
CLN10	Serum	Normal	N/A	1158	37	0.01	5	3.32	0.12
SL04	Serum	Autoimmune	Lupus	11901	19	0.07	38	76.86	3.08
SL07	Serum	Autoimmune	Lupus	626629	25	3.46	150	226.67	8.08
SL08	Serum	Autoimmune	Lupus	4572	15	0.03	52	35.77	2.26
SL09	Serum	Autoimmune	Lupus	560655	29	3.10	148	227.45	8.19
CSLE2	Serum	Autoimmune	Lupus	3996	39	0.02	98	18.02	0.03
CSLE4	Serum	Autoimmune	Lupus	601155	44	3.32	142	155.73	4.57
CSLE5	Serum	Autoimmune	Lupus	80602	19	0.45	90	17.69	0.44
CSLE7	Serum	Autoimmune	Lupus	580654	23	3.21	146	124.13	3.17
CSLE8	Serum	Autoimmune	Lupus	69859	36	0.39	126	106.37	1.38
CSLE10	Serum	Autoimmune	Lupus	173586	22	0.96	142	88.19	2.24
CSLE11	Serum	Autoimmune	Lupus	209119	25	1.15	140	154.59	4.58
CSLE13	Serum	Autoimmune	Lupus	3387	18	0.02	88	5.56	0.24
CSLE14	Serum	Autoimmune	Lupus	22757	45	0.13	33	63.84	1.61
CSLE15	Serum	Autoimmune	Lupus	516340	26	2.85	147	164.33	2.10
SCL02	Serum	Autoimmune	Scleroderma	24708	12	0.14	32	3.73	0.11
SCL05	Serum	Autoimmune	Scleroderma	8260	11	0.05	13	23.55	0.91
SCL07	Serum	Autoimmune	Scleroderma	27780	24	0.15	71	N/A	0.67
SCL11	Serum	Autoimmune	Scleroderma	21056	22	0.12	35	59.46	1.81
SCL14	Serum	Autoimmune	Scleroderma	1089200	15	6.02	145	163.45	2.47
SCL35	Serum	Autoimmune	Scleroderma	1868	15	0.01	N/A	21.16	N/A

Table [SEQ Table * ARABIC]. Clinical Demographics Data

Human Clinical Samples				Gender	Age
Sample ID	Matrix	Species	Strain		
SL04	Serum	Autoimmune	Lupus	Female	33
SL07	Serum	Autoimmune	Lupus	Female	31
SL08	Serum	Autoimmune	Lupus	Male	60
SL09	Serum	Autoimmune	Lupus	Female	40
CSLE2	Serum	Autoimmune	Lupus	Female	27
CSLE4	Serum	Autoimmune	Lupus	Female	20
CSLE5	Serum	Autoimmune	Lupus	Female	57
CSLE7	Serum	Autoimmune	Lupus	Female	42
CSLE8	Serum	Autoimmune	Lupus	Female	22
CSLE10	Serum	Autoimmune	Lupus	Female	38
CSLE11	Serum	Autoimmune	Lupus	Female	45
CSLE13	Serum	Autoimmune	Lupus	Female	45
CSLE14	Serum	Autoimmune	Lupus	Female	33
CSLE15	Serum	Autoimmune	Lupus	Female	32
SCL02	Serum	Autoimmune	Scleroderma	Female	63
SCL05	Serum	Autoimmune	Scleroderma	Female	42
SCL07	Serum	Autoimmune	Scleroderma	Female	63
SCL11	Serum	Autoimmune	Scleroderma	Female	56
SCL14	Serum	Autoimmune	Scleroderma	Female	65
SCL35	Serum	Autoimmune	Scleroderma	Female	75
H8	Serum	Interference Serum	HAMA	Male	21
H14	Serum	Interference Serum	HAMA	Female	32
H16	Serum	Interference Serum	HAMA	Male	42
H17	Serum	Interference Serum	HAMA	Male	28
H18	Serum	Interference Serum	HAMA	Male	55
R21	Serum	Autoimmune	RF	Male	50
R22	Serum	Autoimmune	RF	Male	57
R24	Serum	Autoimmune	RF	Male	59
R25	Serum	Autoimmune	RF	Male	59
R12	Serum	Autoimmune	RF	Female	87

2.12 Assay Summary

Table [SEQ Table * ARABIC]. Assay Summary

Capture Antibody	Arotec native RNP (Sm-free) antigen (Cat# ATR04-05) @ 5 µg/mL in CBC
Coating	Direct Coat
Wash Buffer	1X Enzo from 20X
Assay Buffer	Blocking Buffer (3% BSA, 0.05% Sodium Azide in TBS)
Detector Antibody	Novus Biologicals (Cat# NB100-2046) clone 2C11 @ 100 ng/mL in Theranos AP Conjugate Stabilizer
Detector Stabilizer	Theranos Alkaline Phosphatase Conjugate Stabilizer
Sample Dilution	25x
Post Sample Wash	YES
Edison Protocol	Generic2_25x_PSW_svn_5735

2.13 Reference

- Theranos Laboratory Notebook # 408
- Theranos Experiment Log #E0817