theranos™
redefining healthcare

Anti –SSB (La) Extractable Nuclear Antigen (ENA) Antibody (IgG) Qualitative Assay (In-house Binder) Development Report

Theranos, Inc.

March 11, 2013

Prepared by: Karen Shaw

theranos™
redefining healthcare

TABLE OF CONTENTS

[TOC \O "1-3" \H \Z \U]**LIST OF TABLES**

[TOC \h \z \c "Table"]

LIST OF FIGURES

[TOC \h \z \c "Figure"]

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-SSB (La) (ENA) antibodies (IgG) in human plasma and serum. This assay was previously developed using a commercial capture antigen. This report describes the re-development of the assay using a new in-house SS-B (La) antigen as the capture.

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

The following commercial ELISA kit has been used in-house as a predicate method:

- INOVA Quantalite SSB ELISA (Cat# 708575)

The originally developed Theranos assay with commercial capture antigen has also been used as a reference.

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

SSB (La) antigen (produced in-house) is directly coated on the surface and serves as the capture for the Anti-SSB ENA antibody assay. The sample (plasma or serum) is diluted and then incubated on the capture surface for 10 minutes. The surface is washed, and then an alkaline phosphatase(AP)-labeled anti-human IgG antibody is 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. The resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials

Reagent Name	Supplier	Catalog #
Antigen La (SS-B)	In-house Binders group	n/a
Mouse Anti-Human IgG1 Antibody	Novus Biologicals	NB100-2046
Alkaline Phosphatase Labeling Kit	Dojindo	LK13-10
Phospho Glo Substrate	KPL	55-60-04
Heterophilic Blocking Reagent	Scantibodies, Inc.	3KC533
Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G

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

1.2 Initial Theranos Screen/Training Set

In-house unconjugated SS-B antigen (directly coated on the capture surface), and biotinylated SS-B antigen (coated on an avidin surface) were tested in the Theranos assay along with a commercial SS-B direct-coat surface as a control. Aside from changing the capture antigen, all conditions were set using the originally developed assay conditions, including the protocol (Generic2_10X_PSW). Positive and negative SS-B IgG controls from BioRad were tested, as well as pooled clinical samples, previously confirmed positive or negative in the INOVA assay. The in-house antigens both showed strong response in the assay. The unconjugated antigen provided the highest modulation; however the biotinylated antigen had higher overall signal, indicating possible room for improvement in modulation.

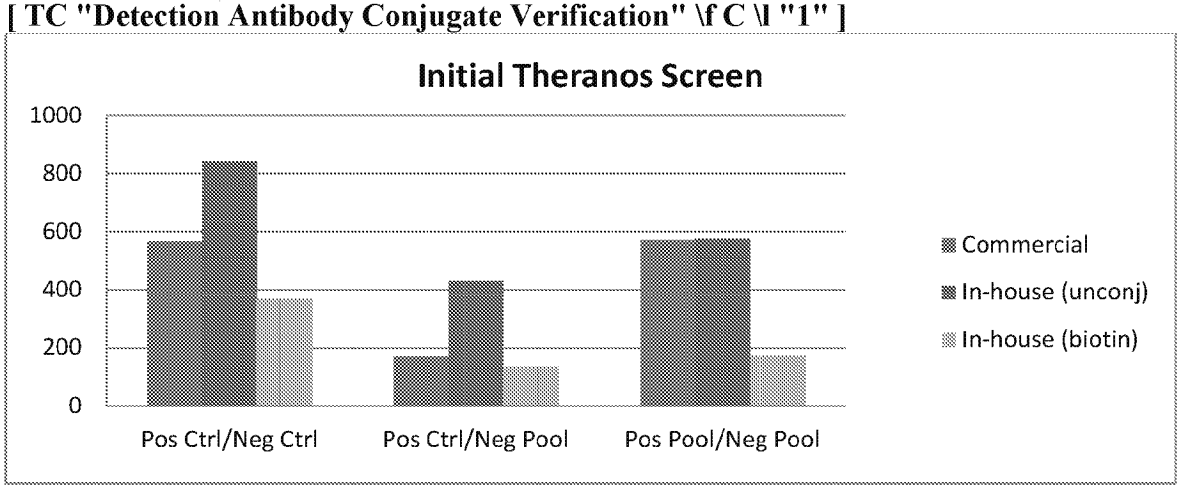
Table [SEQ Table * ARABIC]: Initial Screen RLU

	Commercial Control		In-House Unconjugated		In-House Biotinylated	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	700812	13	1948427	10	2829627	10
Negative Clinical Pool	1224	42	3387	19	16313	11
Positive BioRad Control	209184	11	1456261	12	2221149	2
Negative BioRad Control	369	29	1729	12	6017	12

Table [SEQ Table * ARABIC]: Initial Screen Modulation

	Commercial Control	In-House Unconjugated	In-House Biotinylated
Pos Ctrl/Neg Ctrl	566	842	369
Pos Ctrl/Neg Pool	171	430	136
Pos Pool/Neg Pool	572	575	173

Figure [SEQ Figure * ARABIC]: Initial Screen Modulation
[TC "Detection Antibody Conjugate Verification" \f C \l "1"]



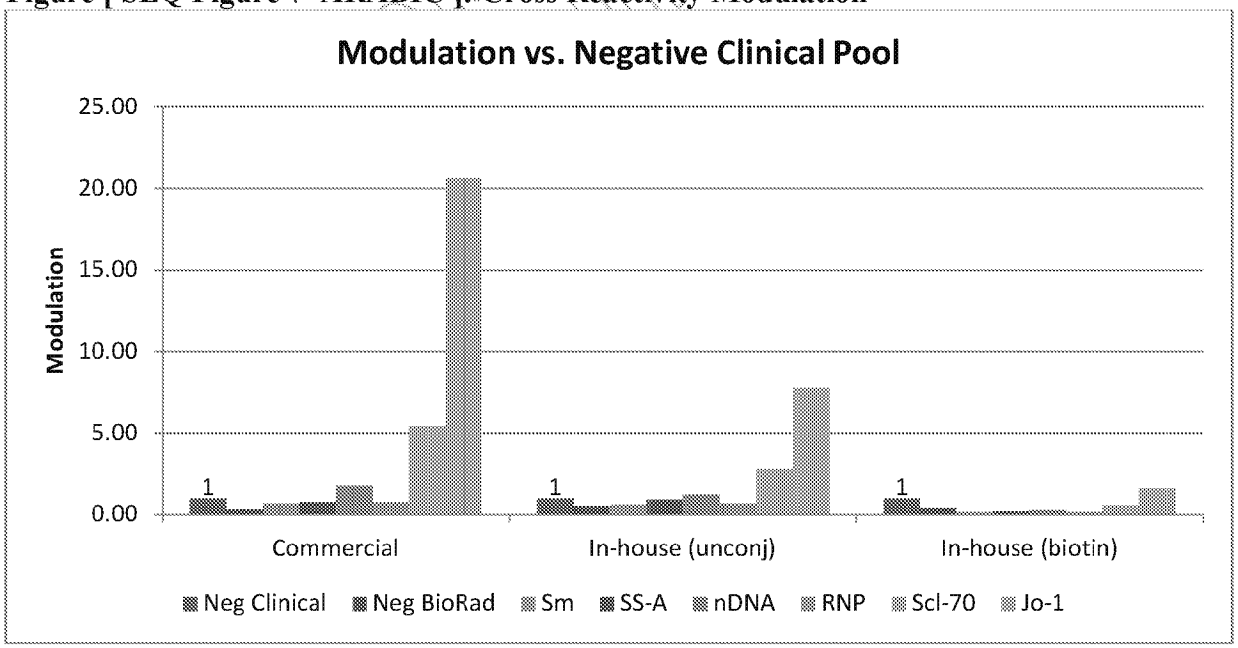
1.3 Cross Reactivity

The in-house capture surfaces were both tested for cross-reactivity with antibodies (IgG) to Sm, SS-A, nDNA, RNP, Scl-70, and Jo-1. All capture surfaces had some cross-reactivity with Jo-1 IgG. The in-house biotinylated capture antigen surface showed the least cross-reactivity overall and was selected for optimization.

Table [SEQ Table * ARABIC]: Cross Reactivity

	Commercial Control			In-House Unconjugated			In-House Biotinylated		
Sample	Mean RLU	CV	Modulation/ Neg. Pool	Mean RLU	CV	Modulation/ Neg. Pool	Mean RLU	CV	Modulation/ Neg. Pool
Sm	842	11	0.7	1981	12	0.6	3424	4	0.2
SS-A	941	10	0.8	3101	5	0.9	7411	15	0.5
nDNA	2162	8	1.8	4186	7	1.2	5670	4	0.3
RNP	939	17	0.8	2287	6	0.7	3871	8	0.2
Scl-70	6660	24	5.4	9496	10	2.8	6975	2	0.4
Jo-1	25236	6	20.6	26399	6	7.8	23841	5	1.5

Figure [SEQ Figure * ARABIC]: Cross Reactivity Modulation



1.4 Capture Antigen Titration

The in-house biotinylated SS-B antigen was coated on an avidin surface at 5, 2.5, and 1 µg/mL. Positive/negative clinical pools and controls were run in the assay. Coating at a concentration of 1 ug/mL provided the best modulation in the assay.

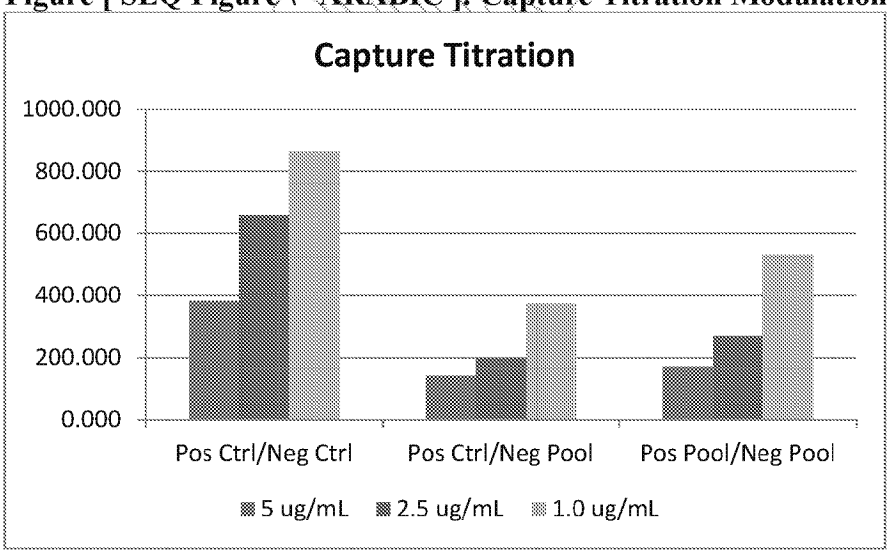
Table [SEQ Table * ARABIC]: Capture Titration RLU

	5 µg/mL		2.5 µg/mL		1.0 µg/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	2677702	5	2400966	20	1641606	17
Negative Clinical Pool	15603	9	8911	30	3088	21
Positive BioRad Control	2202797	19	1744152	7	1155391	21
Negative BioRad Control	5764	12	2644	3	1337	2

Table [SEQ Table * ARABIC]: Capture Titration Modulation

	5 µg/mL	2.5 µg/mL	1.0 µg/mL
Pos Ctrl/Neg Ctrl	382	660	864
Pos Ctrl/Neg Pool	141	196	374
Pos Pool/Neg Pool	172	269	532

Figure [SEQ Figure * ARABIC]: Capture Titration Modulation



1.5 Effect of Coating Buffer

In-house biotinylated SS-B antigen was coated on an avidin surface at 1 µg/mL in StartingBlock, SuperBlock, and SeaBlock blocking buffers. Positive/negative clinical pools and controls were run in the assay and compared to original results using in-house 3% BSA blocking buffer. The in-house 3% BSA blocking buffer provided the best modulation and was selected as the coating buffer.

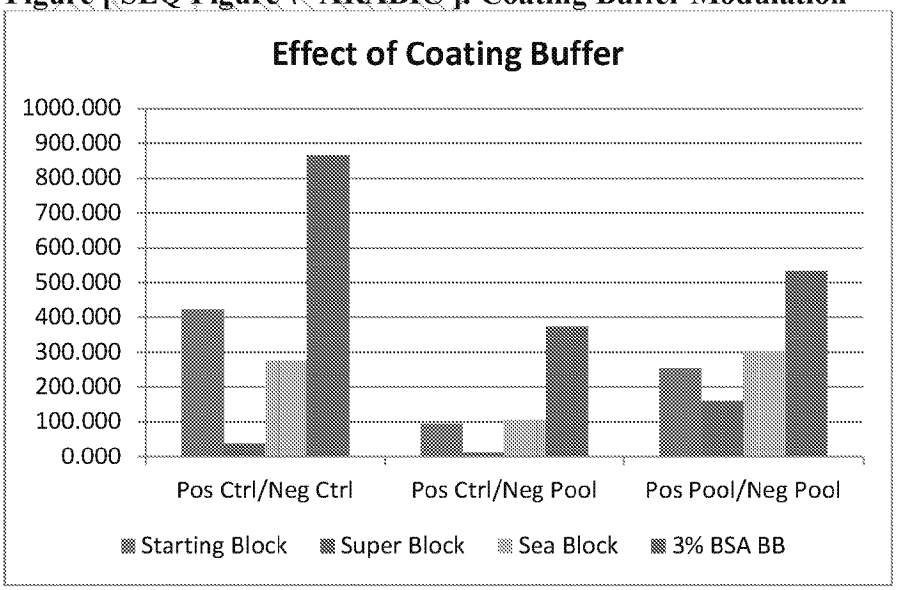
Table [SEQ Table * ARABIC]: Coating Buffer RLU

	StartingBlock		SuperBlock		SeaBlock		3% BSA BB	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	2229007	13	670880	49	1057479	28	1641606	17
Negative Clinical Pool	8767	20	4171	24	4171	37	3088	21
Positive BioRad Control	832748		73551		362164		1155391	21
Negative BioRad Control	1968	6	1267	17	1312	9	1337	2

Table [SEQ Table * ARABIC]: Coating Buffer Modulation

	StartingBlock	SuperBlock	SeaBlock	3% BSA BB
Pos Ctrl/Neg Ctrl	423	58	276	864
Pos Ctrl/Neg Pool	95	18	87	374
Pos Pool/Neg Pool	254	161	254	532

Figure [SEQ Figure * ARABIC]: Coating Buffer Modulation



1.6 Effect of Detection Stabilizer

Detection antibody was diluted to 50 ng/mL in three different AP stabilizers: Theranos in-house, BioStab, and Stabilzyme. Positive/negative clinical pools and controls were run in the assay. The Stabilzyme AP stabilizer provided significantly better modulation between positive and negative clinical samples, and was selected as the detection stabilizer.

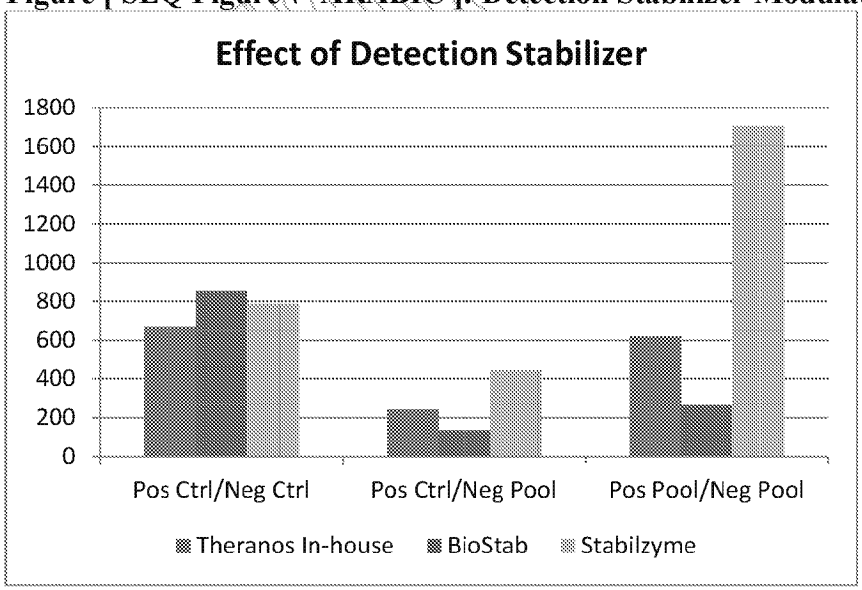
Table [SEQ Table * ARABIC]: Detection Stabilizer RLU

	Theranos In-house		BioStab		Stabilzyme	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	2284851	16	2427227	21	715379	21
Negative Clinical Pool	3690	21	9189	6	419	2
Positive BioRad Control	899616	15	1243199	26	186055	44
Negative BioRad Control	1338	56	1453	7	237	27

Table [SEQ Table * ARABIC]: Detection Stabilizer Modulation

	Theranos In-house	BioStab	Stabilzyme
Pos Ctrl/Neg Ctrl	673	856	787
Pos Ctrl/Neg Pool	244	135	444
Pos Pool/Neg Pool	619	264	1706

Figure [SEQ Figure * ARABIC]: Detection Stabilizer Modulation



1.7 Detection Antibody Titration

Detection antibody was diluted to 200, 100, 50 and 25 ng/mL in Stabilzyme AP stabilizer. Positive/negative clinical pools and controls were run in the assay. 50 ng/mL detection antibody provided the best modulation between positive and negative clinical samples.

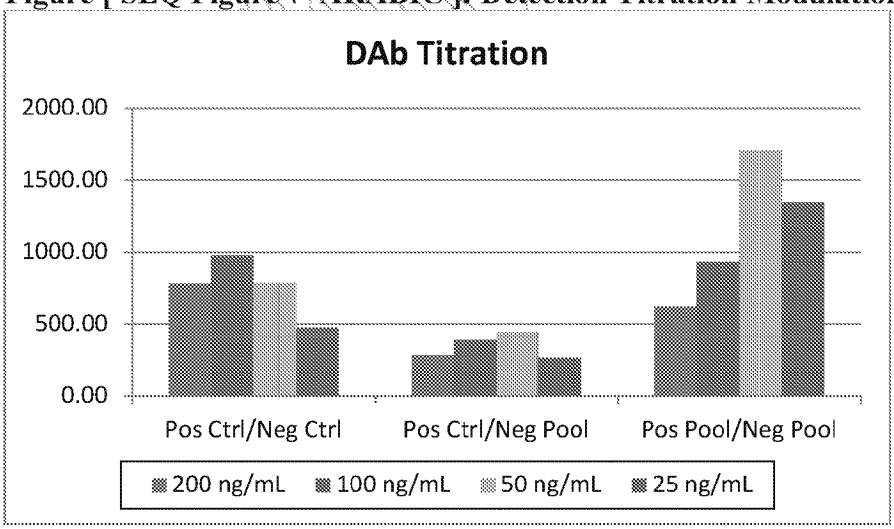
Table [SEQ Table * ARABIC]: Detection Titration RLU

	200 ng/mL		100 ng/mL		50 ng/mL		25 ng/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	2087956	12	1372356	7	715379	21	575266	27
Negative Clinical Pool	3368	9	1471	18	419	2	428	34
Positive BioRad Control	948393	20	572282	9	186055	44	113342	24
Negative BioRad Control	1213	17	586	12	237	27	239	12

Table [SEQ Table * ARABIC]: Detection Titration Modulation

	200 ng/mL	100 ng/mL	50 ng/mL	25 ng/mL
Pos Ctrl/Neg Ctrl	782	977	787	474
Pos Ctrl/Neg Pool	282	389	444	265
Pos Pool/Neg Pool	620	933	1706	1346

Figure [SEQ Figure * ARABIC]: Detection Titration Modulation



1.8 Effect of HBR in Assay Diluent

Assay diluent was spiked with heterophilic blocking reagent (HBR) at 400 µg/mL and 200 µg/mL, and compared with un-spiked assay diluent. Positive/negative clinical pools and controls were run in the assay. 400 µg/mL HBR provided the best modulation between positive and negative clinical samples.

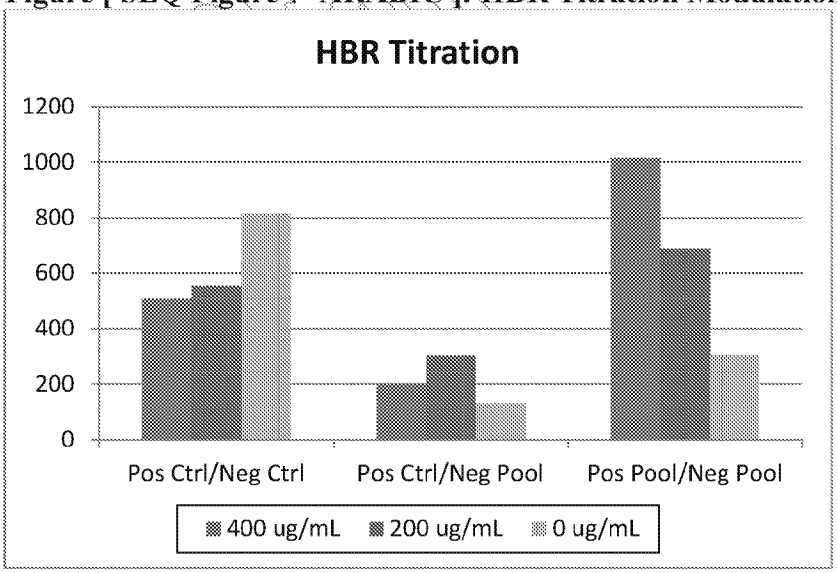
Table [SEQ Table * ARABIC]: HBR Titration RLU

	HBR 400 µg/mL		HBR 200 µg/mL		HBR 0 µg/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	801496	18	668074	10	611325	18
Negative Clinical Pool	791	45	971	17	1999	8
Positive BioRad Control	156531	17	294458	48	260400	5
Negative BioRad Control	309	26	530	76	320	13

Table [SEQ Table * ARABIC]: HBR Titration Modulation

	HBR 400 µg/mL	HBR 200 µg/mL	HBR 0 µg/mL
Pos Ctrl/Neg Ctrl	507	555	814
Pos Ctrl/Neg Pool	198	303	130
Pos Pool/Neg Pool	1014	688	306

Figure [SEQ Figure * ARABIC]: HBR Titration Modulation



1.9 Effect of Protocol (Sample Dilution and Incubation Times)

Positive/negative clinical pools and controls were run in the assay with varying protocols. First, sample dilutions of 10X, 25X and 50X were tested. 25X was selected due to fewer high CVs.

Next, incubation times of 10-10-10 minutes and 5-5-5 minutes were tested, as well as a 10-10-10 protocol with no post-sample wash (PSW). Modulation was best with the 10-10-10 minute protocol with PSW.

Table [SEQ Table * ARABIC]: Sample Dilution RLU

	10X		25X		50X	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	801496	18	935237	16	863647	33
Negative Clinical Pool	791	45	1061	8	829	55
Positive BioRad Control	156531	17	229706	5	109674	29
Negative BioRad Control	309	26	306	21	578	8

Table [SEQ Table * ARABIC]: Sample Dilution Modulation

	10X	25X	50X
Pos Ctrl/Neg Ctrl	507	750	190
Pos Ctrl/Neg Pool	198	217	132
Pos Pool/Neg Pool	1014	882	1041

Table [SEQ Table * ARABIC]: Incubation Times and PSW RLU

	10-10-10 PSW		5-5-5 PSW		10-10-10 no PSW	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	935237	16	309815	3	677890	16
Negative Clinical Pool	1061	8	532	6	1315	22
Positive BioRad Control	229706	5	46946	15	210470	19
Negative BioRad Control	306	21	424	4	1199	40

Table [SEQ Table * ARABIC]: Incubation Times and PSW Modulation

	10-10-10 PSW	5-5-5 PSW	10-10-10 no PSW
Pos Ctrl/Neg Ctrl	750	111	175
Pos Ctrl/Neg Pool	217	88	160
Pos Pool/Neg Pool	882	582	516

1.10 Clinical Sample Screen

Thirty normal plasma samples previously confirmed negative for SS-B IgG in the INOVA kit were tested in the assay to establish a cut-off. All samples had very low RLUs as expected; however the CVs were very high. Five confirmed positive SS-B IgG clinical samples were also screened, and CVs were still unacceptably high even at the higher RLU levels.

Table [SEQ Table * ARABIC]: Negative Clinical Samples

Sample	Mean RLU	CV	Sample	Mean RLU	CV
1	1004	9	SA07	966	33
2	814	18	SA08	2032	40
3	719	16	SA09	753	13
4	850	28	SA10	1250	10
5	942	14	SA11	644	2
6	2142	16	SA12	5283	37
7	659	20	SA13	849	38
8	875	37	SA14	895	27
9	1365	25	SA15	3862	23
10	1164	8	SA16	1680	10
11	1780	30	SA17	1424	7
12	1070	11	SA18	659	8
13	660	11	SA19	906	15
14	774	14	SA20	909	10
15	1298	25	SA21	5994	14

Table [SEQ Table * ARABIC]: Positive Clinical Samples

Sample	Mean RLU	CV
SL04	99447	60
SL06	753174	16
CSLE7	1150970	24
SS03	522434	61
SS09	22429	24

1.11 CV Troubleshooting

To resolve the CV problem, the in-house unconjugated SS-B capture antigen was re-examined. A fresh coating was performed with the in-house biotinylated SS-B on an avidin surface at 1 µg/mL, and the unconjugated in-house and commercial directly on the surface at 5 µg/mL. Positive/negative clinical pools and controls were run, with 18 replicates tested for each condition. The in-house unconjugated antigen had the lowest overall CVs in the assay.

Next, the in-house unconjugated SS-B antigen was coated at 10, 5, and 2.5 µg/mL. Positive and negative controls were run, 18 replicates per condition. Increasing capture concentration led to improved CVs. This may explain why CVs with biotinylated antigen increased when coating concentration was set at 1 µg/mL. Modulation and CVs were best with in-house unconjugated antigen at 10 µg/mL. This was selected as the new capture surface, with all further optimization experiments to be repeated on this new surface.

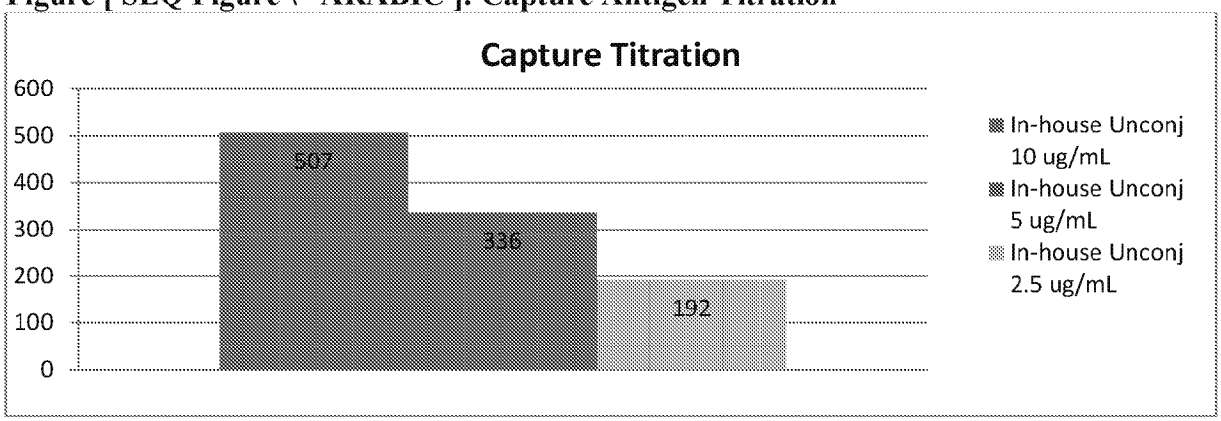
Table [SEQ Table * ARABIC]: CV Troubleshooting 1

	In-house Bt 1 µg/mL		In-house Unconj. 5 µg/mL		Commercial Unconj. 5 µg/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	1531395	20	722956	9	153739	12
Negative Clinical Pool	1113	32	814	17	376	22
Positive BioRad Control	495439	39	295657	14	29769	37
Negative BioRad Control	829	36	603	13	264	48

Table [SEQ Table * ARABIC]: CV Troubleshooting 2

	In-house Unconj. 10 µg/mL		In-house Unconj. 5 µg/mL		In-house Unconj. 2.5 µg/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive BioRad Control	545214	8	345908	11	111697	29
Negative BioRad Control	1075	16	1028	38	581	22

Figure [SEQ Figure * ARABIC]: Capture Antigen Titration



1.12 Effect of Detection Stabilizer - Unconjugated Capture

Detection antibody was diluted to 50 ng/mL in three different AP stabilizers: Theranos in-house, BioStab, and Stabilzyme. Positive/negative clinical pools and controls were run in the assay. The Stabilzyme AP stabilizer provided better modulation between positive and negative clinical samples, and was selected as the detection stabilizer.

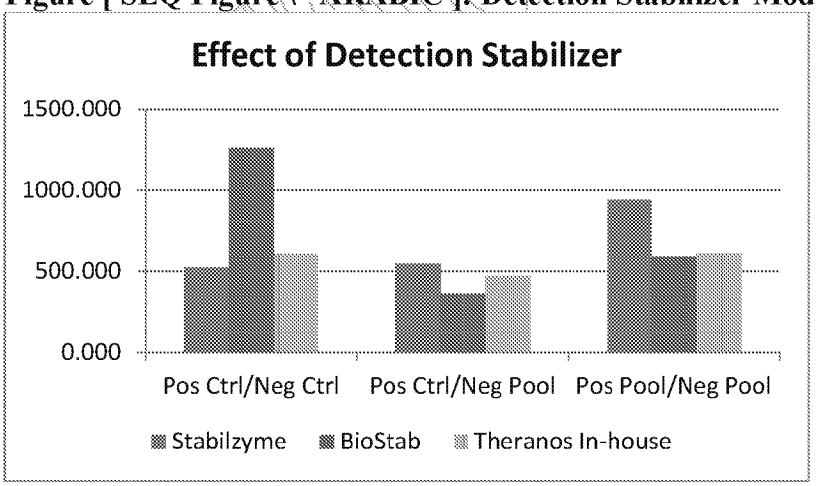
Table [SEQ Table * ARABIC]: Detection Stabilizer RLU

	Stabilzyme		BioStab		Theranos In-house	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	1001170	4	2022549	7	1867492	8
Negative Clinical Pool	1168	12	3420	15	3054	18
Positive BioRad Control	574396	9	1238237	4	1445622	4
Negative BioRad Control	1241	18	979	55	2374	1

Table [SEQ Table * ARABIC]: Detection Stabilizer Modulation

	Stabilzyme	BioStab	Theranos In-house
Pos Ctrl/Neg Ctrl	525	1265	609
Pos Ctrl/Neg Pool	548	362	473
Pos Pool/Neg Pool	944	591	611

Figure [SEQ Figure * ARABIC]: Detection Stabilizer Modulation



1.13 Detection Antibody Titration - Unconjugated Capture

Detection antibody was diluted to 200, 100, 50 and 25 ng/mL in Stabilzyme AP stabilizer. Positive/negative clinical pools and controls were run in the assay. 50 ng/mL detection antibody was selected due to having consistently lower intra- and inter-CVs, while still providing acceptable modulation.

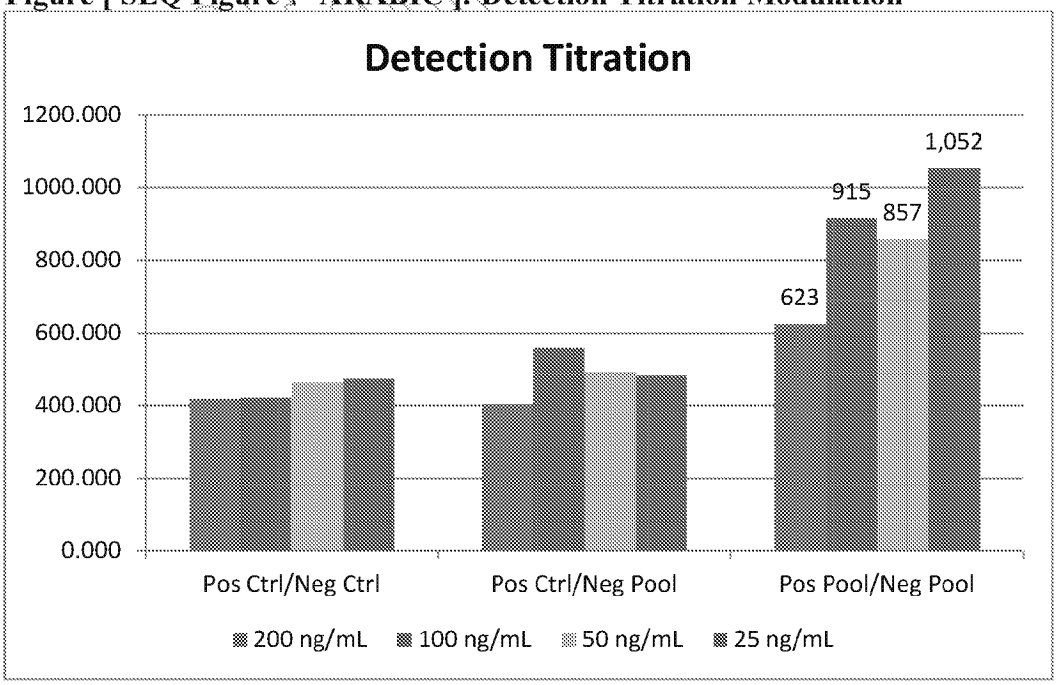
Table [SEQ Table * ARABIC]: Detection Titration RLU

	200 ng/mL		100 ng/mL		50 ng/mL		25 ng/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	2436601	17	1697098	9	1001170	4	690371	5
Negative Clinical Pool	3909	37	1855	8	1168	12	656	10
Positive BioRad Control	1578893	6	1033755	3	574396	9	316279	9
Negative BioRad Control	3779	3	2460	10	1241	18	669	31

Table [SEQ Table * ARABIC]: Detection Titration Modulation

	200 ng/mL	100 ng/mL	50 ng/mL	25 ng/mL
Pos Ctrl/Neg Ctrl	418	420	463	473
Pos Ctrl/Neg Pool	404	557	492	482
Pos Pool/Neg Pool	623	915	857	1052

Figure [SEQ Figure * ARABIC]: Detection Titration Modulation



1.14 Effect of HBR in Assay Diluent - Unconjugated Capture

Assay diluent was spiked with heterophilic blocking reagent (HBR) at 400 µg/mL and 200 µg/mL, and compared with un-spiked assay diluent. Positive/negative clinical pools and controls were run in the assay. 200 µg/mL HBR provided the best modulation between positive and negative clinical samples. Assay conditions were fixed as: 10 µg/mL unconjugated SS-B capture antigen, 50 ng/mL detection antibody in Stabilzyme, and assay diluent with 200 ng/mL HBR.

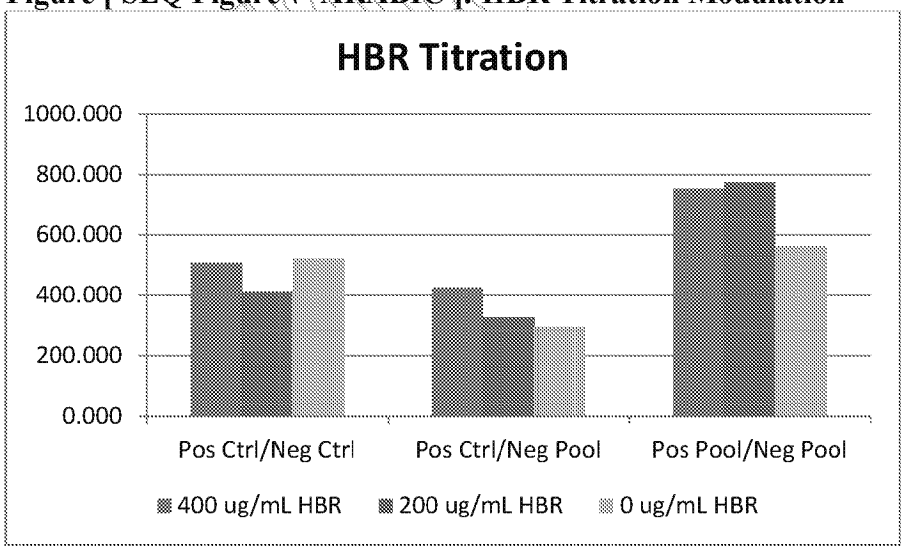
Table [SEQ Table * ARABIC]: HBR Titration RLU

	HBR 400 µg/mL		HBR 200 µg/mL		HBR 0 µg/mL	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	1151662	17	1383670	7	1421496	19
Negative Clinical Pool	1530	13	1784	11	2520	19
Positive BioRad Control	651387	7	586067	19	749607	14
Negative BioRad Control	1284	10	1416	11	1437	9

Table [SEQ Table * ARABIC]: HBR Titration Modulation

	HBR 400 µg/mL	HBR 200 µg/mL	HBR 0 µg/mL
Pos Ctrl/Neg Ctrl	507	414	522
Pos Ctrl/Neg Pool	426	329	297
Pos Pool/Neg Pool	753	776	564

Figure [SEQ Figure * ARABIC]: HBR Titration Modulation



1.15 Effect of Protocol - Unconjugated Capture

Positive/negative clinical pools and controls were run in the assay with varying protocols. First, sample dilutions of 10X, 25X and 50X were tested. 25X provided the best modulation of clinical samples.

Next, incubation times of 10-10-10 minutes and 5-5-5 minutes were tested, as well as a 10-10-10 protocol with no post-sample wash (PSW). Modulation was best with the 10-10-10 minute protocol with PSW. The protocol was finalized as Generic2_25X_PSW.

Table [SEQ Table * ARABIC]: Sample Dilution RLU

	10X		25X		50X	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	1147485	12	1396568	2	1264906	4
Negative Clinical Pool	2388	6	1712	9	1778	18
Positive BioRad Control	954612	7	695945	13	418593	11
Negative BioRad Control	1616	5	1589	23	1505	27

Table [SEQ Table * ARABIC]: Sample Dilution Modulation

	10X	25X	50X
Pos Ctrl/Neg Ctrl	591	438	278
Pos Ctrl/Neg Pool	400	407	235
Pos Pool/Neg Pool	481	816	712

Table [SEQ Table * ARABIC]: Incubation Times and PSW RLU

	10-10-10 PSW		5-5-5 PSW		10-10-10 no PSW	
Sample	Mean RLU	CV	Mean RLU	CV	Mean RLU	CV
Positive Clinical Pool	1390119	8	456330	3	772675	12
Negative Clinical Pool	1802	9	841	8	1576	10
Positive BioRad Control	646000	17	180434	11	712541	10
Negative BioRad Control	1503	18	748	13	1460	14

Table [SEQ Table * ARABIC]: Incubation Times and PSW Modulation

	10-10-10 PSW	5-5-5 PSW	10-10-10 no PSW
Pos Ctrl/Neg Ctrl	430	241	488
Pos Ctrl/Neg Pool	358	215	452
Pos Pool/Neg Pool	771	543	490

1.16 Negative Clinical Sample Screen - Finalized Conditions

Thirty normal plasma samples previously confirmed negative for SS-B IgG in the INOVA kit were tested in the assay to establish a cut-off. Preliminary cut-off for the assay was set as 7 standard deviations above the mean negative RLU, with clinical designations based on signal/cut-off (S/CO) ratio. See table 34 for preliminary cut-off information.

Table [SEQ Table * ARABIC]: Negative Clinical Samples

Sample	Mean RLU	CV	Sample	Mean RLU	CV
1	1706	14	SA07	1577	10
2	1699	21	SA08	1561	10
3	1619	8	SA09	1454	14
4	1479	3	SA10	1605	13
5	1927	6	SA11	1393	6
6	1307	18	SA12	76993	24
7	1264	3	SA13	1303	17
8	1536	4	SA14	1218	12
9	1401	15	SA16	1770	4
10	1538	15	SA17	1403	5
11	2287	14	SA18	1431	10
12	1539	22	SA19	1326	3
13	1820	8	SA20	1494	6
14	1768	12	SA21	1836	18
15	1379	1	SA07	1577	10

Table [SEQ Table * ARABIC]: Preliminary Cut-off Determination

Overall Mean RLU	4063
Std Dev	13776
Cut-off (=7*StdDev+Mean)	100496
S/CO >1	Positive
S/CO 0.9-1	Equivocal
S/CO <0.9	Negative

1.17 Positive Clinical Sample Screen – Finalized Conditions

Fifteen SS-B IgG positive clinical samples were screened in both the Theranos assay (using the aforementioned preliminary cut-off) and the INOVA predicate assay. Correlation of results between methods was acceptable. The in-house unconjugated SS-B antigen may now be used as capture surface in the SS-B IgG assay under these finalized conditions.

Table [SEQ Table * ARABIC]: Positive Clinical Samples

Sample	Mean RLU	CV	Theranos S/CO	INOVA Result
SS05	1265825	7	12.60	108.31
SS09	163644	17	1.63	39.73
CSLE6	460629	17	4.58	70.50
CSLE7	1402534	15	13.96	126.92
SL01	1046730	13	10.42	107.04
SL04	160314	7	1.60	40.63
SL06	1040619	8	10.35	109.78
CSLE4	15028	15	0.15	27.69
SS03	718152	8	7.15	100.56
SJ03	274987	11	2.74	59.63
SJ04	143065	18	1.42	42.31
SJ05	138095	11	1.37	42.25

INOVA key		Tentative Theranos Key	
>80	Strong Positive	>1	Positive
40-80	Moderate Positive	0.9-1	Equivocal
20-39	Weak Positive	<0.9	Negative
<20	Negative		

1.18 Reproducibility

Three separate lots of in-house SS-B antigen were produced and coated on tips at 10 µg/mL. Positive/negative clinical pools and controls were run in the finalized assay, as well as five individual SS-B IgG negative clinical samples and five SS-B IgG positive clinical samples. The previously established cut-off from lot 1 was used to calculate all results. All three lots performed comparably, with results matching the known state of the samples and controls.

Table [SEQ Table * ARABIC]: Reproducibility

	Lot 1			Lot 2			Lot 3		
Sample	Mean RLU	CV	S/CO	Mean RLU	CV	S/CO	Mean RLU	CV	S/CO
Pos. Clinical Pool	495879	13.1	4.9	1295092	14.3	12.9	1482990	10.6	14.8
Neg. Clinical Pool	488	16.1	0.0	2016	13.2	0.0	3338	7.1	0.0
Pos. BioRad Ctrl	268289	25.3	2.7	601411	13.7	6.0	624822	12.2	6.2
Neg. BioRad Ctrl	424	36.2	0.0	1076	13.0	0.0	1581	13.5	0.0
Normal 7	418	9.8	0.0	2063	5.1	0.0	2906	7.1	0.0
Normal 8	524	22.8	0.0	1436	9.7	0.0	1964	6.1	0.0
Normal 9	617	15.0	0.0	4893	4.0	0.0	6470	20.1	0.1
Normal 10	710	26.6	0.0	3811	11.2	0.0	4891	15.9	0.0
Normal 11	996	16.2	0.0	1976	24.2	0.0	2764	9.6	0.0
Positive SS03	415587	24.9	4.1	766089	6.7	7.6	905212	6.0	9.0
Positive SS05	645466	14.4	6.4	1240699	9.6	12.3	1437796	9.2	14.3
Positive CSLE6	221521	12.8	2.2	430595	2.2	4.3	407631	13.5	4.1
Positive CSLE7	553555	5.5	5.5	1241144	9.0	12.4	1436524	8.9	14.3
Positive SL06	432221	43.4	4.3	995142	17.8	9.9	1220704	17.8	12.1

1.18 Stability Studies

Stability of reagents at was tested to 12 weeks. Positive and negative controls were run in the assay at each time-point. Results were comparable at all time-points tested, indicating the assay reagents are stable for at least 12 weeks when stored at 4°C.

Table [SEQ Table * ARABIC]: Stability

	Mean RLU				
Control	Day 1	Week 1	Week 4	Week 8	Week 12
Positive	330254	296273	273060	335515	272025
Negative	451	332	500	581	623

Figure [SEQ Figure * ARABIC]: Stability