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Thyroid Stimulating Hormone (TSH) 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 detect human Thyroid Stimulating Hormone (TSH) in human whole blood, plasma and serum. The assay has a reportable range of 0.05 to 50 uIU/mL, and is calibrated to the WHO Third International Standard (NIBSC 81/565).

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:

- Alpco TSH ELISA (Cat# 25-TSHHU-E01)
- Alpco Ultrasensitive TSH ELISA (Cat # 25-TSHHUUE01)
- Genway TSH ELISA (Cat #40-521-475018)

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

A biotin-labeled anti-TSH antibody coated on an avidin surface serves as the capture surface for the sandwich ELISA. The sample (whole blood, plasma or serum) is diluted and then incubated on the capture surface for 10 minutes, then an alkaline phosphatase-labeled anti-TSH antibody is incubated on the surface for 10 minutes. After the detection antibody incubation, the surface is washes and the alkaline phosphatase substrate is incubated on the surface for 10 minutes, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #
Human TSH	Cell Sciences	CRT504-100
Mouse Anti-Human TSH Antibody (CAb)	US Biological	T5400-13D
Mouse Anti-Human TSH Antibody (DAb)	US Biological	T5400-22G
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13
Biotin Labeling Kit (SH)	Dojindo	LK10
Phospho Glo Substrate	KPL	55-60-04
Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G
Carbonate-bicarbonate buffer	Sigma	C3041

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

1.2 Antibody Screening (MTP)[TC "Detection Antibody Conjugate Verification" \f C \l "1"]

To determine the optimal pair for the TSH ELISA, all combinations of 22 TSH antibodies were tested on a microtitre plate. The screening was performed with serum calibrators diluted 1:5 into assay buffer, 10ug/mL CAb and 100ng/mL detection antibody in blocking buffer and no post sample wash.

Table [SEQ Table * ARABIC]: Antibody Information

Number	Vendor	Cat #	Clone #	Type
1	Thermo Scientific	MA1-82909	155	Mab
2	Thermo Scientific	MA1-83492	183	Mab
3	Thermo Scientific	MA1-82908	154	Mab
4	MyBiosource	MBS530254	157183	Mab
5	Usbiological	T5400-43A	9F349	MAb
6	Usbiological	T5400-49	3F366	MAb
7	Usbiological	T5400-13A	5E807	MAb
8	Usbiological	T5400-13D	9L710	MAb
9	Usbiological	T5400-13K	9L716	MAb
10	Usbiological	T5400-43D	9F352	MAb
11	Usbiological	T5400-43E	9F353	MAb
12	Usbiological	T5400-43F	10F570	MAb
13	Usbiological	T5400-43M	10F571	MAb
14	Usbiological	T5400-22E	10F572	Mab
15	Usbiological	T5400-22G	10F574	Mab
16	Usbiological	T5400-47	1.B.168	MAb
17	Usbiological	T5400-28	n/a	Goat PAb
18	Usbiological	T5400-22D	9L717	Mab
19	Usbiological	T5400-43Q	10K373	Mab
20	R&D Systems	MAB57941	512908	MAb
21	Calbiotech	TSCS249	Not provided	MAb
22	Calbiotech	TSCS346	Not provided	MAb

Table [SEQ Table * ARABIC]: Summary of Antibody Screening Results

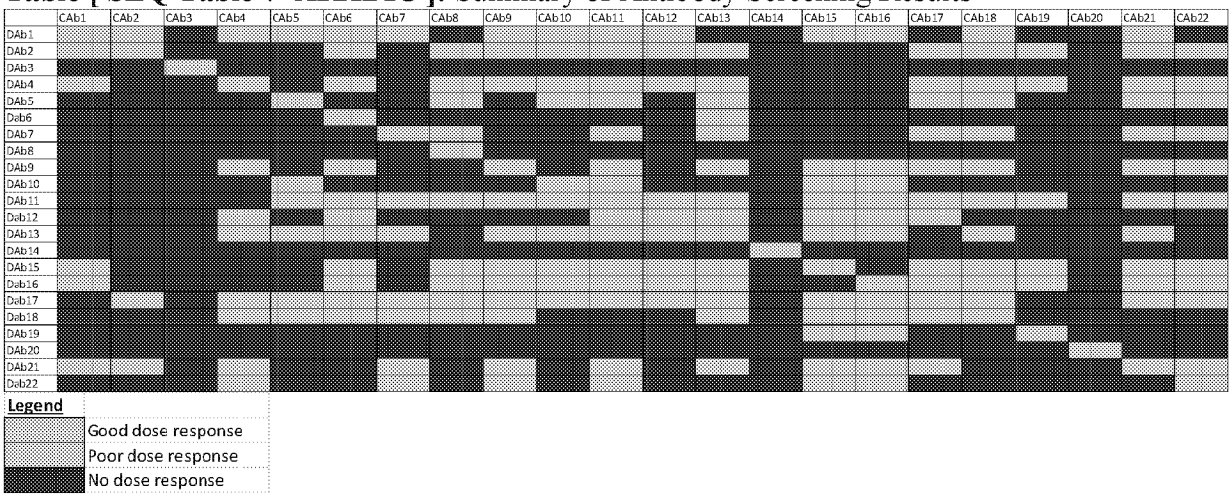


Table [SEQ Table * ARABIC]: Summary of Best Pairs (MTP)

Dab	Cab	[TSH] uIU/mL	Mean RLU	CV %	Modulation
1	2	25	42151	1.5	125
		10	18113	7.5	54
		5	9831	7.3	29
		0	338	13.6	
1	16	25	47127	3.8	142
		10	20325	0.4	61
		5	10805	4.1	33
		0	332	33.8	
2	1	25	78645	7.0	197
		10	34546	4.3	87
		5	17500	1.7	44
		0	398	2.2	
2	8	25	53692	1.9	132
		10	20729	2.5	51
		5	11743	7.4	29
		0	406	6.4	
2	9	25	53124	1.1	133
		10	21687	11.5	54
		5	11905	1.3	30
		0	398	8.0	
2	13	25	59055	0.3	140
		10	23977	2.4	57
		5	13724	0.2	32
		0	423	19.8	

Dab	Cab	[TSH] uIU/mL	Mean RLU	CV %	Modulation
2	17	25	60266	1.9	292
		10	23797	2.5	115
		5	13710	0.1	66
		0	206	7.0	
2	22	25	83205	6.5	198
		10	34682	0.5	82
		5	19927	3.4	47
		0	421	9.6	
4	1	25	61511	9.2	184
		10	24947	4.0	75
		5	14225	16.1	43
		0	334	7.8	
4	8	25	43568	1.7	147
		10	16341	1.9	55
		5	9825	1.0	33
		0	297	20.5	
4	13	25	47462	9.5	154
		10	18103	7.0	59
		5	10112	13.3	33
		0	309	27.2	
4	17	25	49107	4.6	205
		10	17876	0.3	75
		5	10455	0.9	44
		0	239	25.4	
4	22	25	61953	3.0	198
		10	26158	6.5	84
		5	14000	4.3	45
		0	313	6.5	
15	1	25	112600	3.8	255
		10	40711	7.0	92
		5	23914	4.3	54
		0	441	2.7	
15	8	25	65250	1.2	142
		10	25503	1.1	55
		5	16533	5.4	36
		0	460	18.6	
15	9	25	79018	3.4	178
		10	32122	3.8	73

Dab	Cab	[TSH] uIU/mL	Mean RLU	CV %	Modulation
		5	18810	0.6	42
		0	443	19.3	
15	12	25	56724	0.4	196
		10	22025	7.1	76
		5	12717	2.6	44
		0	289	5.1	
15	13	25	82499	3.2	184
		10	31244	4.3	70
		5	17230	2.2	38
		0	449	13.1	
15	17	25	95230	1.3	329
		10	34539	2.4	119
		5	19270	6.3	67
		0	289	5.1	
15	22	25	104788	1.4	202
		10	42741	2.6	82
		5	23697	1.7	46
		0	520	13.6	
16	1	25	43978	7.6	217
		10	18068	0.6	89
		5	9873	0.6	49
		0	203	21.4	
16	8	25	30195	0.8	140
		10	11672	11.7	54
		5	7467	3.8	35
		0	215	1.3	
16	12	25	22883	2.6	157
		10	8447	2.7	58
		5	4908	0.8	34
		0	145	6.0	
16	13	25	33218	4.3	131
		10	12447	6.2	49
		5	7266	3.8	29
		0	254	0.0	
16	17	25	35968	3.8	262
		10	13594	0.0	99
		5	7678	0.0	56
		0	137	19.0	

Dab	Cab	[TSH] uIU/mL	Mean RLU	CV %	Modulation
16	22	25	46046	2.9	162
		10	18099	2.3	64
		5	10348	2.0	36
		0	285	17.3	
17	2	25	104191	4.0	448
		10	34896	3.0	150
		5	23687	4.3	102
		0	233	16.3	
17	4	25	94265	0.3	360
		10	37646	0.2	144
		5	20562	2.2	79
		0	262	7.8	
17	5	25	52909	8.1	127
		10	17822	1.9	43
		5	11658	4.0	28
		0	418	7.7	
17	6	25	42796	4.2	256
		10	18767	8.2	112
		5	10090	5.7	60
		0	167	12.2	
17	9	25	81460	9.9	168
		10	31321	2.6	65
		5	18996	0.6	39
		0	484	11.4	
17	12	25	46521	1.0	184
		10	17408	1.6	69
		5	10556	0.4	42
		0	253	8.0	
17	15	25	90769	3.0	324
		10	38124	3.0	136
		5	21841	3.3	78
		0	280	6.2	
17	16	25	99963	0.5	385
		10	40946	1.6	158
		5	22216	0.8	86
		0	260	9.0	

1.3 Cross Reactivity and Interference (MTP)

TSH is a glycoprotein comprised of an alpha and a beta subunit. The alpha subunit is identical to that of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Human chorionic gonadotropin (hCG). The beta subunit confers specificity among these pituitary hormones. Due to this identical alpha subunit, the potential for cross reactivity is extremely high. Diagnosis of hyperthyroidism involves low levels of TSH, while FSH, LH or hCG could be elevated, so it is essential that the chosen antibody pair is free of cross reactivity that could lead to a false-normal measurement when in fact TSH is depressed. Therefore, cross reactivity and interference testing was conducted as a part of the antibody screening process.
Most of the pairs tested showed cross reactivity with one or all of the other hormones tested when no TSH was present. Borderline cross reactivity was defined as greater than 115% of the control background RLU, while unacceptable cross reactivity was defined as greater than 125% of the control background RLU. The candidate pairs with borderline or no cross reactivity of the microtitre plate were chosen for a cross reactivity screen on the Theranos System.

The assay conditions were with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. Calibrators were prepared for TSH in TSH-depleted serum at 2x concentration, and then combined with equal volumes of either serum (control) or 2x test substance-spiked depleted serum to create 1x serum calibrators. The final concentration of the test substances in the serum were as follows:

FSH at 562.5 ng/mL (900mIU/mL)
LH at 60 ng/mL
hCG at 10,000 ng/mL

Table [SEQ Table * ARABIC]: Cross Reactivity and Interference (MTP)

			Control		Spiked with FSH			Spiked with LH			Spiked with hCG		
DAb	CAb	[TSH] uIU/mL	Mean RLU	CV %	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL
17	2	25	57830	0.4	56845	0.5	98	54045	1.1	93	50164	3.4	87
		10	22674	3.9	20623	2.6	91	19925	0.5	88	18880	2.6	83
		5	12717	2.7	11948	2.9	94	10987	4.5	86	11123	9.5	87
		2.5	6434	3.8	6365	5.3	99	5328	5.2	83	5579	5.7	87
		1.25	4145	1.1	4325	7.5	104	3528	2.9	85	3689	2.3	89
		0	236	4.9	757	8.4	321	426	23.7	180	326	15.9	138
17	16	25	152746	3.2	144364	1.6	95	127773	3.2	84	135638	3.4	89
		10	69324	3.9	57604	10.0	83	49882	1.6	72	52120	3.8	75
		5	33975	5.9	30640	2.1	90	26865	0.0	79	28136	8.6	83
		2.5	17127	2.3	15144	2.6	88	12807	1.0	75	12923	1.8	75
		1.25	9912	4.8	9366	9.7	94	8332	2.0	84	8143	3.0	82
		0	481	14.8	580	2.9	121	656	30.4	136	384	0.7	80
17	4	25	86173	0.0	83184	2.2	97	80258	2.1	93	79745	3.2	93

			Control		Spiked with FSH			Spiked with LH			Spiked with hCG		
DAb	CAb	[TSH] uIU/mL	Mean RLU	CV %	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL
		10	33695	0.3	31331	4.3	93	29812	2.4	88	30974	2.4	92
		5	18295	1.8	17935	1.9	98	16737	0.4	91	17486	4.6	96
		2.5	9294	0.6	8841	1.8	95	8277	0.6	89	8440	2.3	91
		1.25	5079	0.6	5415	4.7	107	5288	1.8	104	5167	0.6	102
		0	310	14.7	419	9.5	135	443	5.1	143	348	7.4	112
15	17	25	60027	5.9	55191	1.4	92	52669	2.0	88	56489	3.9	94
		10	25164	0.5	22253	2.9	88	20387	4.3	81	21603	3.8	86
		5	12871	0.7	12732	1.2	99	10673	2.2	83	11473	1.6	89
		2.5	6420	2.2	6366	0.8	99	5627	1.4	88	5641	5.4	88
		1.25	3896	4.2	3963	4.5	102	3315	2.6	85	3360	12.9	86
		0	174	16.4	723	19.4	415	356	12.9	205	233	33.2	134
17	15	25	115133	2.1	119970	0.7	104	114755	4.6	100	108516	2.7	94
		10	48877	2.7	44993	1.7	92	42910	1.8	88	44817	2.1	92
		5	25154	4.4	24502	1.4	97	23852	1.5	95	23868	4.4	95
		2.5	12706	3.4	13776	2.6	108	13036	1.2	103	12578	3.1	99
		1.25	7767	0.7	7575	6.4	98	7459	4.4	96	7693	6.5	99
		0	348	-	782	10.5	225	706	10.0	203	594	17.6	171
2	17	25	57830	0.4	56845	0.5	98	54045	1.1	93	50164	3.4	87
		10	22674	3.9	20623	2.6	91	19925	0.5	88	18880	2.6	83
		5	12717	2.7	11948	2.9	94	10987	4.5	86	11123	9.5	87
		2.5	6434	3.8	6365	5.3	99	5328	5.2	83	5579	5.7	87
		1.25	4145	1.1	4325	7.5	104	3528	2.9	85	3689	2.3	89
		0	236	4.9	757	8.4	321	426	23.7	180	326	15.9	138
15	1	25	63564	1.4	63788	4.1	100	60080	0.6	95	57864	0.8	91
		10	25465	1.8	21848	1.8	86	20288	0.5	80	22408	4.7	88
		5	14002	5.9	13487	4.4	96	12796	8.0	91	13273	0.4	95
		2.5	7801	1.7	6373	3.3	82	6745	4.4	86	6542	7.6	84
		1.25	4166	1.9	4001	2.4	96	4146	1.9	100	4039	11.7	97
		0	469	3.7	356	3.2	76	596	22.5	127	539	23.8	115
16	1	25	80109	0.2	77623	1.6	97	77513	2.3	97	77973	1.8	97
		10	32129	1.3	32091	0.2	100	31834	0.5	99	33898	0.9	106
		5	19177	4.4	17321	5.5	90	17633	0.2	92	18685	2.8	97
		2.5	10389	0.4	9394	3.6	90	9430	8.2	91	9716	5.1	94
		1.25	5754	0.3	5087	3.8	88	5188	2.9	90	5467	3.9	95
		0	423	11.9	352	11.1	83	694	5.2	164	725	55.9	171
15	22	25	149040	0.9	135046	2.0	91	121850	0.9	82	124379	2.1	83
		10	57907	1.9	55415	2.2	96	53961	0.5	93	53374	2.0	92

			Control		Spiked with FSH			Spiked with LH			Spiked with hCG		
DAb	CAb	[TSH] uIU/mL	Mean RLU	CV %	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL	Mean RLU	CV %	% of CTL
		5	32418	1.1	30023	2.3	93	28014	0.9	86	26175	2.2	81
		2.5	16932	5.9	15704	0.4	93	14884	6.7	88	14509	0.3	86
		1.25	9272	1.5	8819	0.1	95	8139	2.8	88	7559	0.2	82
		0	505	20.2	670	13.5	133	686	8.3	136	600	11.8	119
2	1	25	113706	3.5	103842	2.6	91	104079	2.4	92	105040	0.1	92
		10	43035	0.7	43447	1.3	101	42217	0.1	98	43157	2.6	100
		5	23711	4.2	21667	1.1	91	22716	4.2	96	23220	1.2	98
		2.5	11949	0.1	11763	10.6	98	11439	0.7	96	13497	11.7	113
		1.25	6665	2.0	6289	1.2	94	6479	3.9	97	6742	3.9	101
		0	390	0.7	439	1.3	113	610	25.0	156	483	-	124
15	12	25	83725	1.1	78095	0.1	93	69699	0.5	83	72060	2.2	86
		10	31086	2.5	30711	1.1	99	29190	5.7	94	29066	2.7	94
		5	16623	1.1	15605	2.3	94	15948	3.0	96	17174	5.6	103
		2.5	8459	0.5	8421	6.6	100	8461	4.4	100	8732	0.3	103
		1.25	4633	0.5	4424	1.3	95	4504	1.2	97	4508	0.9	97
		0	355	1.6	303	3.7	85	391	-	110	383	-	108
15	13	25	108918	0.3	97092	0.4	89	93022	0.5	85	100577	2.4	92
		10	42586	2.6	42291	0.9	99	39543	1.3	93	41081	2.6	96
		5	24052	1.0	23194	2.2	96	21988	4.7	91	22758	0.3	95
		2.5	12373	4.2	11301	1.6	91	10643	2.5	86	10821	1.8	87
		1.25	7033	3.2	6691	3.7	95	6106	0.1	87	6280	6.0	89
		0	586	2.0	682	20.9	117	626	-	107	642	3.6	110
15	9	25	74777	3.9	66651	0.3	89	66319	1.3	89	67057	0.4	90
		10	30133	4.4	28774	3.9	95	27911	4.0	93	28176	1.8	94
		5	15643	0.2	14337	0.7	92	15554	2.0	99	14556	3.0	93
		2.5	7798	8.1	8328	8.4	107	7688	2.4	99	7011	0.6	90
		1.25	5067	9.9	4462	1.3	88	4355	5.2	86	4804	19.8	95
		0	487	2.3	479	-	98	527	-	108	515	11.0	106
15	8	25	87387	6.8	75894	2.7	87	76596	0.5	88	82202	1.1	94
		10	33784	9.9	31059	1.4	92	32231	3.8	95	33145	3.5	98
		5	20812	11.9	17601	8.8	85	16978	0.2	82	16869	2.5	81
		2.5	8989	2.3	8402	1.7	93	8101	1.7	90	8443	0.8	94
		1.25	5323	2.6	4965	3.1	93	5331	3.5	100	4963	0.5	93
		0	684	-	615	10.2	90	708	-	104	607	5.7	89

1.4 Theranos System Screen and Cross Reactivity

Cross reactivity was combined with the usual antibody screening on the Theranos System, in order to determine the ideal antibody pair for the final automated assay conditions. The Theranos System test was done with a regular serum standard curve to evaluate the antibody pair dose response, and with the following concentrations of test hormones in the TSH-depleted serum to test for cross reactivity in the absence of TSH under the Theranos System conditions. The assay conditions were DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution.

Concentration of cross reactivity test substances:
FSH at 562.5 ng/mL (900mIU/mL)
LH at 60 ng/mL
hCG at 10,000 ng/mL

From the Theranos System screening, there were 2 pairs that showed a good dose response and negligible cross reactivity with the other hormones: DAb 15 with CAb 8 and DAb 15 with CAb 12. These 2 pairs were chosen as the final candidate pairs to continue on with further testing.

Table [SEQ Table * ARABIC]: Theranos System Screen and Cross Reactivity

		Dose Response (N=3 cartridges)				Cross Reactivity (N=3 cartridges)			
Dab	**Cab**	**[TSH] uIU/mL**	**Mean RLU**	**CV %**	**Modulation**	**Test Substance**	**Mean RLU**	**CV %**	**% of CTL**
17	16	25.00	711094	3.9	244	CTL	2432	12.0	
		10.00	422091	12.9	145	FSH	2845	4.7	**117**
		5.00	237781	15.0	81	LH	3583	6.1	**147**
		2.50	129320	10.0	44	hCG	2858	3.4	**117**
		1.25	68661	11.1	24				
		0.00	2920	14.3					
15	1	25.00	332995	13.0	218	CTL	1368	6.7	
		10.00	180400	9.9	118	FSH	1425	4.1	104
		5.00	84110	4.6	55	LH	1921	3.3	**140**
		2.50	47993	5.4	31	hCG	1579	16.6	**115**
		1.25	24926	7.0	16				
		0.00	1526	5.2					
15	8	25.00	293908	5.4	146	CTL	1909	12.1	
		10.00	119532	19.5	59	FSH	2055	28.7	108
		5.00	57890	6.2	29	LH	2005	16.5	105
		2.50	29056	8.1	14	hCG	1688	21.8	88
		1.25	16273	11.7	8				
		0.00	2012	9.0					

Table 6: Theranos System Screen and Cross Reactivity, Continued

		Dose Response (N=3 cartridges)				Cross Reactivity (N=3 cartridges)			
Dab	**Cab**	**[TSH] uIU/mL**	**Mean RLU**	**CV %**	**Modulation**	**Test Substance**	**Mean RLU**	**CV %**	**% of CTL**
15	9	25.00	276158	17.0	152	CTL	1677	1.0	
		10.00	124090	9.0	69	FSH	2066	2.1	**123**
		5.00	64173	23.2	35	LH	2208	7.0	**132**
		2.50	31326	22.3	17	hCG	1868	11.3	111
		1.25	16936	19.3	9				
		0.00	1811	8.6					
15	12	25.00	184624	16.4	138	CTL	1353	0.9	
		10.00	106077	22.2	79	FSH	1365	7.5	101
		5.00	53710	11.5	40	LH	1530	7.1	113
		2.50	29042	8.4	22	hCG	1479	6.8	109
		1.25	14057	14.9	10				
		0.00	1340	3.6					
15	13	25.00	217580	10.0	128	CTL	1477	17.0	
		10.00	107464	5.5	63	FSH	1749	23.7	**118**
		5.00	55351	1.5	33	LH	1817	19.6	**123**
		2.50	30109	13.3	18	hCG	1627	14.5	110
		1.25	18071	20.4	11				
		0.00	1700	14.2					
15	22	25.00	293299	8.1	207	CTL	1478	18.3	
		10.00	156740	22.3	110	FSH	1814	8.0	**123**
		5.00	80490	16.2	57	LH	2029	12.0	**137**
		2.50	40642	7.3	29	hCG	1642	14.1	111
		1.25	21818	10.9	15				
		0.00	1420	5.0					

1.5 Training Set

1.5.1 Training Set Screen for Best 2 Pairs

The final candidate pairs were used to test 11 clinical serum samples on the Theranos System with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. These samples were provided by Bioreclamation with pre-measured concentrations of TSH and were tested in the Alpco TSH ELISA and Alpco Ultrasensitive TSH ELISA, and the clinical correlations were compared. Clinical correlation will be later verified on a larger sample set with the final pair and the final assay conditions on a full calibration curve.

Both pairs of candidate antibodies produced acceptable correlation with both the supplier-reported concentration and the Alpco ELISA measured concentration for this small sample set. However CAb 12 provided better sensitivity with the un-optimized assay conditions.

Table [SEQ Table * ARABIC]: Clinical Samples - Training Set Results

	Abbott Architect (Reported by Bioreclamation)	Alpco Regular Kit	Alpco Ultrasensitive Kit	Genway Kit	Theranos System, [TSH] uIU/mL	
Sample#	[TSH] uIU/mL	[TSH] uIU/mL	[TSH] uIU/mL	[TSH] uIU/mL	CAb 8	CAb 12
3	1.07	1.40	0.96	0.7	0.75	0.92
4	1.58	3.63	1.54	3.1	2.08	2.22
6	1.69	3.40	1.91	1.8	1.87	2.12
12	3.56	5.34	2.57	2.8	4.38	3.08
15	6.94	5.73	3.57	4.1	6.60	5.70
17	0.02	0.27	0.11	0.0	0.06	0.07
18	0.16	0.40	0.34	0.0	0.07	0.18
20	1.20	3.02	1.33	1.0	2.00	1.74
23	3.10	5.42	2.42	2.6	4.55	3.06
24	4.50	8.72	4.64	3.2	6.86	5.50
25	5.40	11.84	8.34	4.5	8.95	6.58

* Bioreclamation reported values were measured before at least 2 freeze-thaw cycles – once by Bioreclamation and once in house for aliquoting of samples. All other measurements were completed after the final aliquoting.

Table [SEQ Table * ARABIC]: Standard Curve in Serum: Pair 1

	Signal (RLU)		Back-Calculated Conc. (uIU/mL)		
[TSH] uIU/mL	Mean RLU	CV %	Mean Conc	CV %	% Recovery
25.00	293908	5.4	25.24	5.5	101
10.00	119532	19.5	10.24	19.6	102
5.00	57890	6.2	4.90	6.4	98
2.50	29056	8.1	2.34	9.0	94
1.25	18518	20.8	1.40	24.4	112
0.63	9925	20.1	0.65	25.8	104
0.31	4982	8.3	0.25	12.0	81
0.16	3977	12.3	0.18	18.5	116
0.00	2308	8.4	0.08	13.9	

Conc = 10^(0.0672*(LOG(RLU))^3 - 1.0483*(LOG(RLU))^2 + 6.45*(LOG(RLU)) - 13.51)

Table [SEQ Table * ARABIC]: Standard Curve in Serum: Pair 2

	Signal (RLU)		Back-Calculated Conc. (uIU/mL)		
[TSH] uIU/mL	Mean RLU	CV %	Mean Conc	CV %	% Recovery
25.00	190363	12.6	26.03	26.2	104
10.00	106077	22.2	9.92	28.2	99
5.00	53710	11.5	4.55	10.8	91
2.50	29042	8.4	2.73	6.5	109
1.25	12487	17.3	1.35	16.7	108
0.63	5706	4.2	0.53	6.5	84
0.31	4222	1.6	0.32	2.9	101
0.16	3080	2.4	0.17	5.5	106
0.00	1256	11.5	0.01	40.1	

Conc = 10^(0.5614*(LOG(RLU))^3 - 7.4616*(LOG(RLU))^2 + 33.826*(LOG(RLU)) - 51.812)

Figure [SEQ Figure * ARABIC]: Correlation of Alpco Regular ELISA to Abbott Architect Result

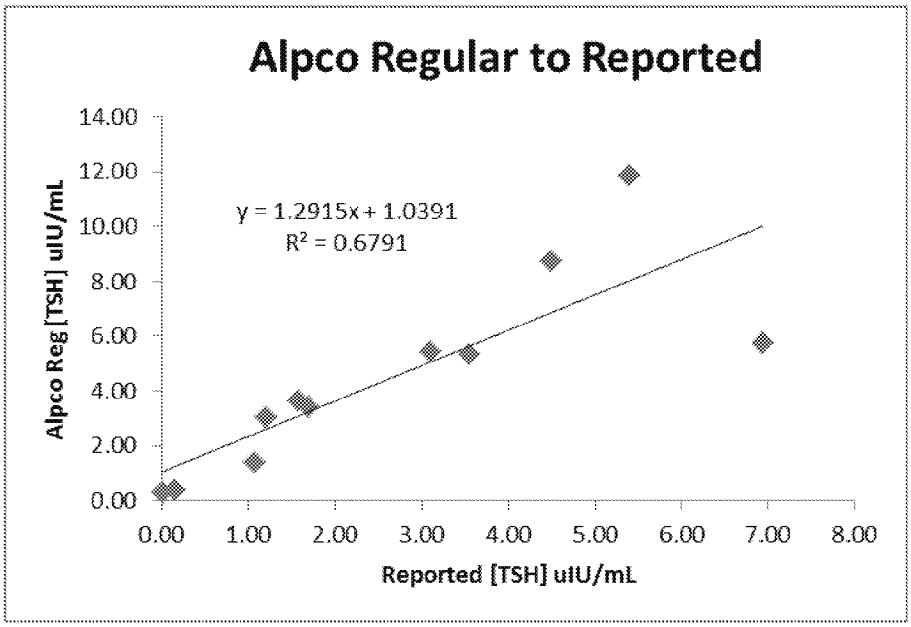


Figure [SEQ Figure * ARABIC]: Correlation of Alpco Ultrasensitive ELISA to Abbott Architect Result

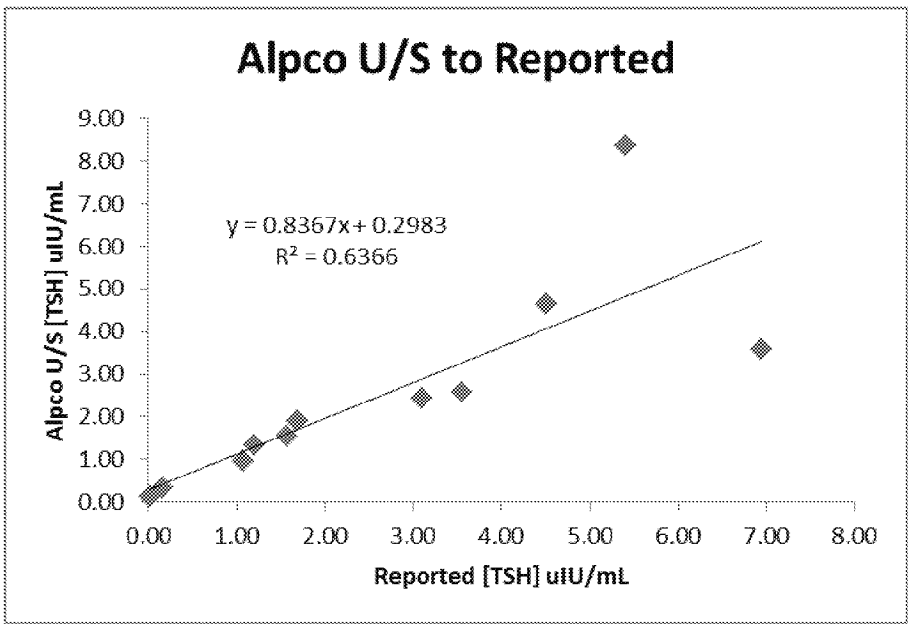


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Abbott Architect Result, Pair 1

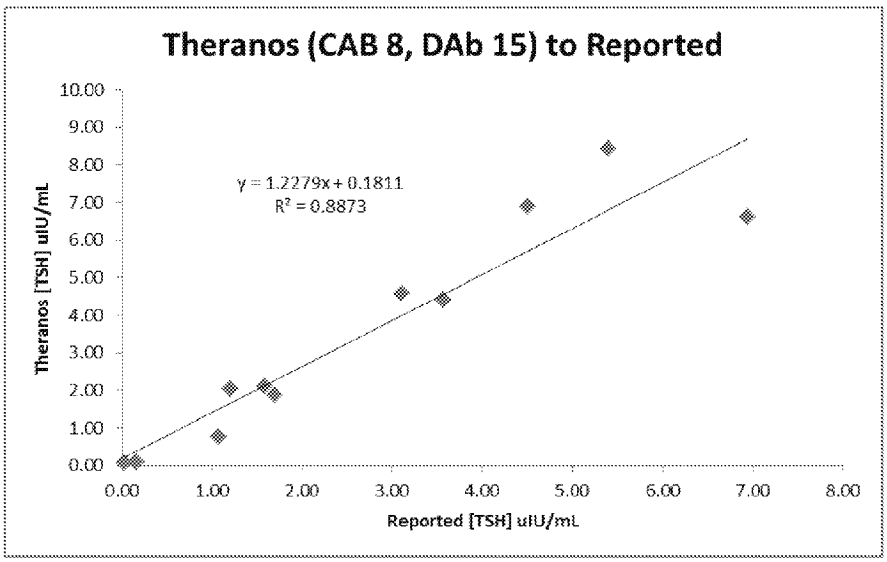


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Abbott Architect Result, Pair 2

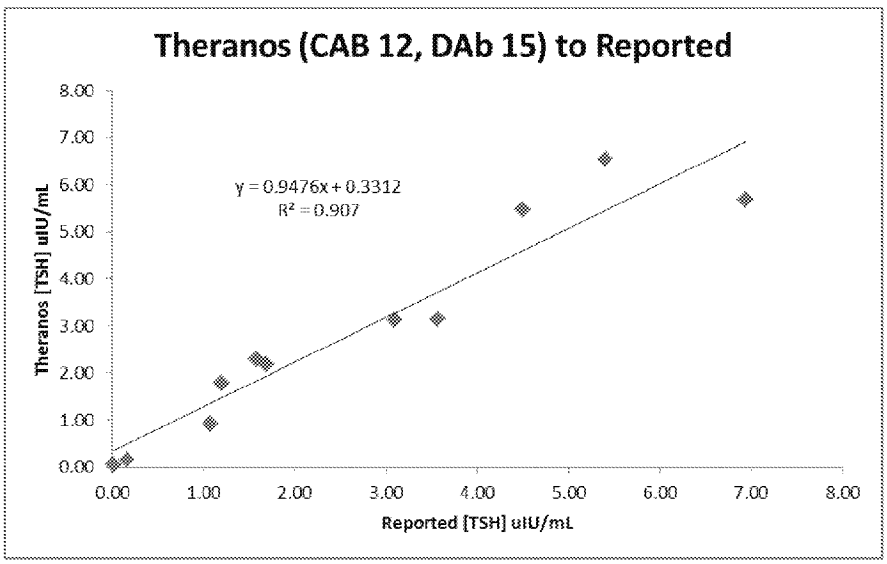


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Alpco Regular ELISA Result, Pair 1

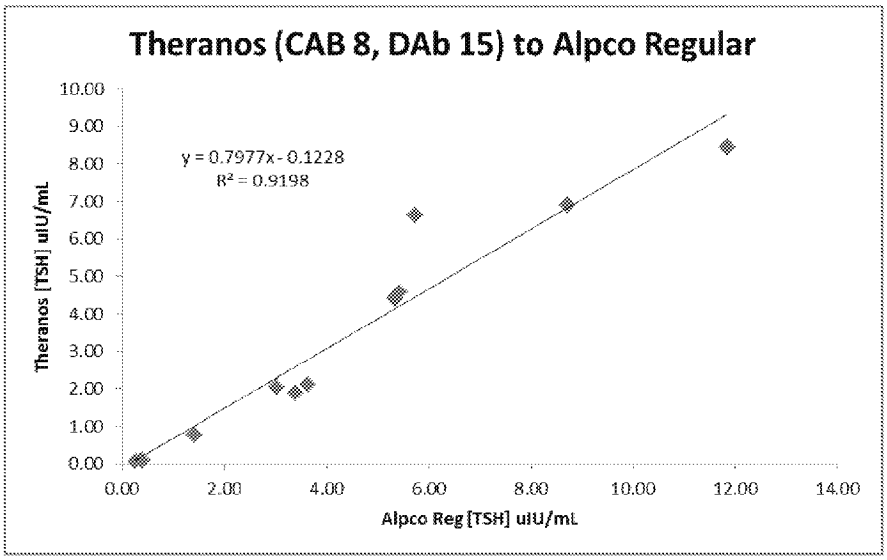


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Alpco Regular ELISA Result, Pair 2

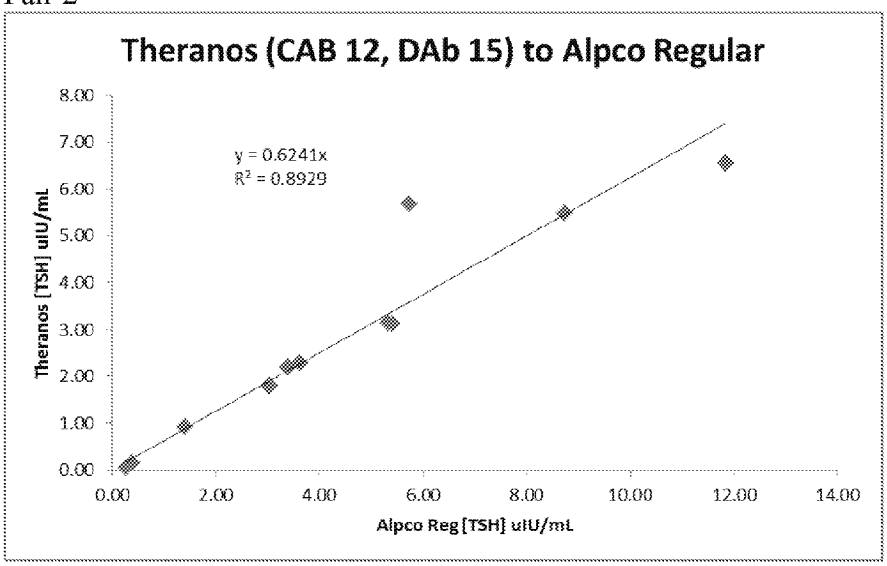


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Alpco Ultrasensitive ELISA Result, Pair 1

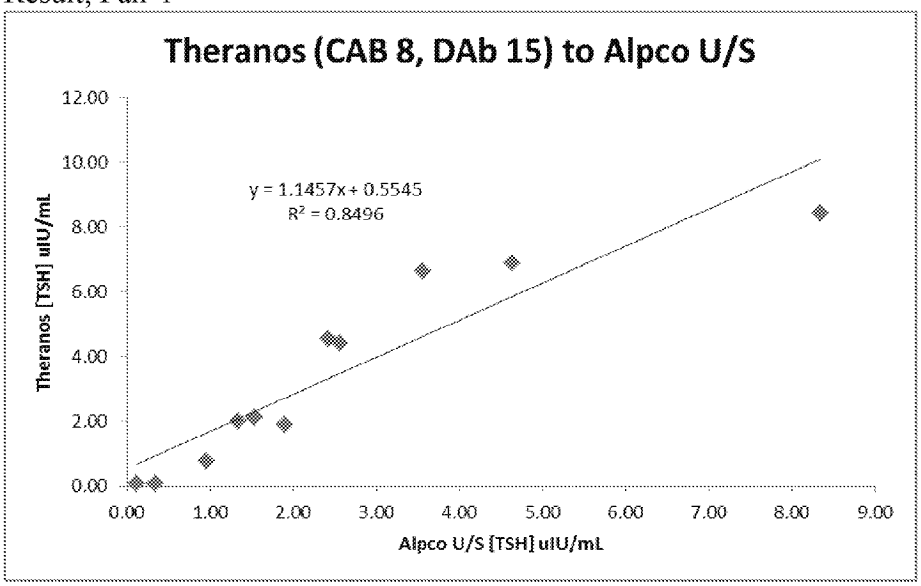


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Alpco Ultrasensitive ELISA Result, Pair 2

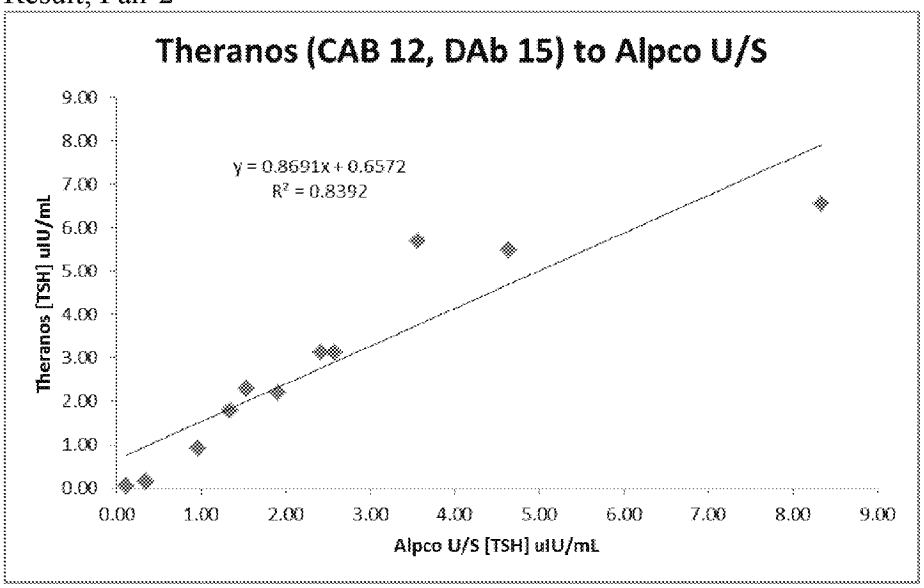


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Genway ELISA Result, Pair 1

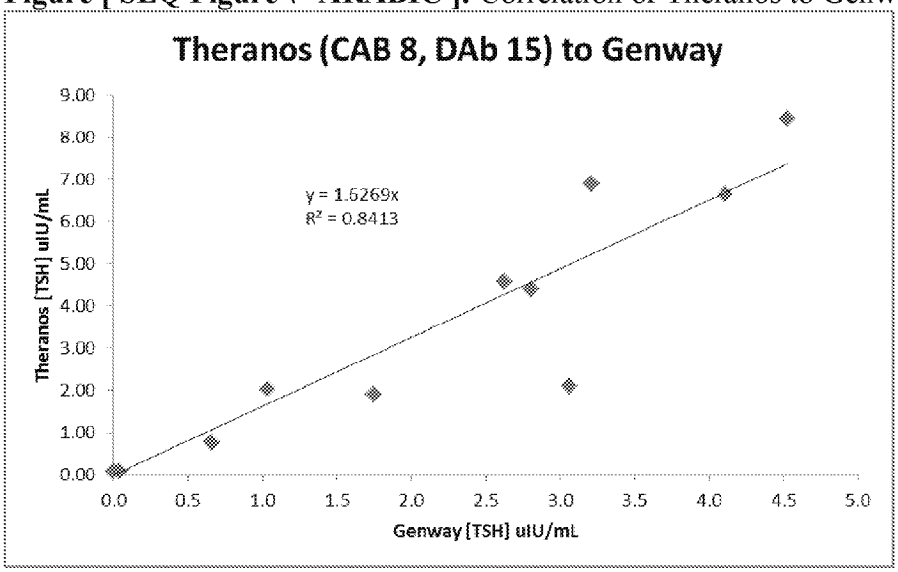
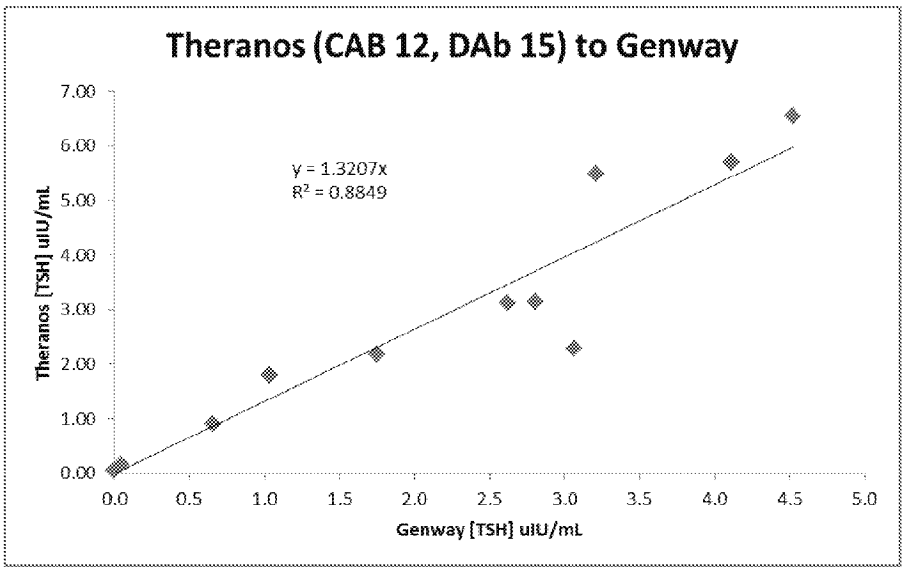


Figure [SEQ Figure * ARABIC]: Correlation of Theranos to Genway ELISA Result, Pair 2



1.5.2 Complete Clinical Set for Final Pair

Once the antibody pair was finalized as CAb 12 and DAb 15, the complete set was compared in the Theranos Assay and the predicate methods. The correlation with the reported values and predicate methods was excellent across the assay range.

Table [SEQ Table * ARABIC]: Clinical Samples – Predicate and Theranos Results, uIU/mL

Sample	Abbott Architect (Bioreclamation Reported)	Alpco Regular Kit	Alpco U/S Kit	Genway	Theranos
1	0.87	1.55		0.78	0.72
2	0.91	1.96		0.59	0.87
3	1.07	1.40	0.96	0.66	0.91
4	1.58	3.63	1.54	3.06	2.28
5	1.64	1.32		0.48	0.85
6	1.69	3.40	1.91	1.75	2.18
7	1.91	3.07		1.38	1.85
8	2.49	2.89		1.66	2.26
9	2.83	2.65		1.83	1.77
10	26.96	40.74		24.00	30.34
11	3.20	5.31		7.87	2.87
12	3.56	5.34	2.57	2.81	3.13
13	5.38	6.43		4.22	4.08
14	6.80	9.83		6.53	6.48
15	6.94	5.73	3.57	4.11	5.69
16	0.30	0.62		0.02	0.29
17	0.02	0.27	0.11	OORL	0.05
18	0.16	0.40	0.34	0.04	0.14
19	0.90	1.64		0.63	0.86
20	1.20	3.02	1.33	1.03	1.79
21	10.90	17.00		7.62	11.40
22	13.90	15.59		10.07	11.40
23	3.10	5.42	2.42	2.62	3.11
24	4.50	8.72	4.64	3.21	5.49
25	5.40	11.84		4.52	6.55

Table [SEQ Table * ARABIC]: Standard Curve

	Signal (RLU)		Back-Calculated Conc. (uIU/mL)		
[TSH] uIU/mL	**Mean RLU**	**CV %**	**Mean**	**CV %**	**% Recovery**
30.00	348489	10.5	29.49	8.5	98
10.00	146179	13.9	10.28	12.2	103
5.00	80562	9.7	5.29	11.4	106
2.50	37552	15.7	2.32	17.7	93
1.00	16368	10.6	0.93	11.2	93
0.50	10400	9.3	0.55	10.0	109
0.20	4836	11.2	0.20	11.2	101
0.05	1912	6.5	0.05	3.0	99
0.00	806	17.1	0.01	42.9	-

Figure [SEQ Figure * ARABIC]: Theranos Result vs Reported Concentration

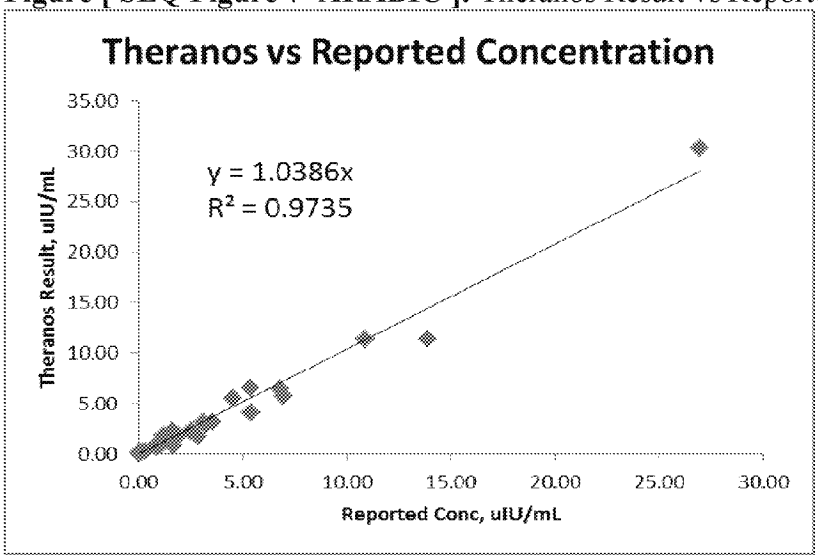


Figure [SEQ Figure * ARABIC]: Theranos toAlpco Regular Kit Result

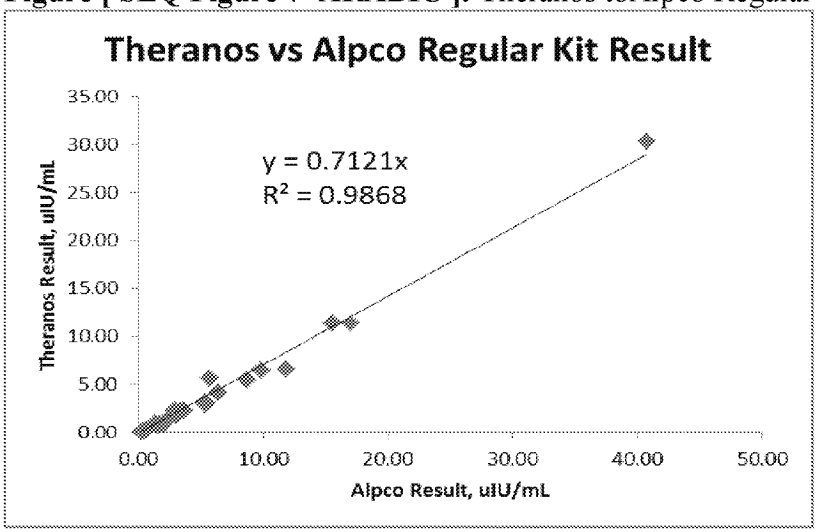


Figure [SEQ Figure * ARABIC]: Theranos to Alpco Ultrasensitive Kit Result

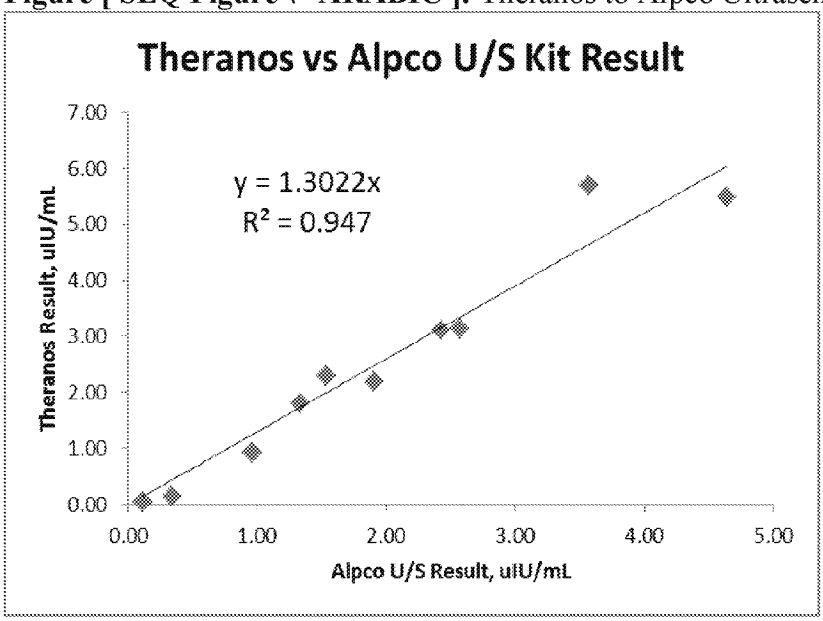
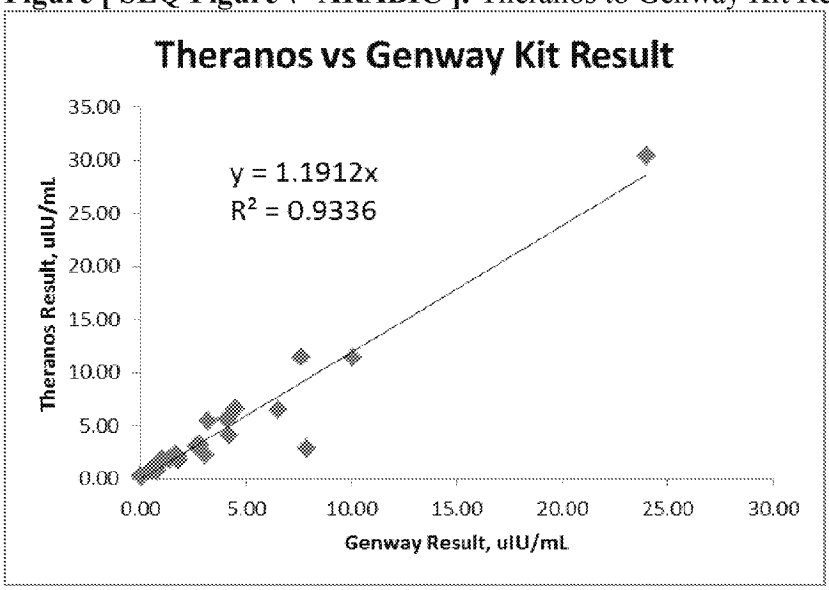


Figure [SEQ Figure * ARABIC]: Theranos to Genway Kit Result



1.6 Matrix Screen and Spike Recovery

To finalize the selection of the best antibody pair, a matrix screen was performed with spiked whole blood, plasma from the same sample of whole blood, serum and assay buffer with with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. Neither pair showed significant matrix effects, but DAb 15 with CAb 12 performed most uniformly in all the tested matrixes. For this experiment, concentrations were all calculated based on the assay buffer standard curve (all other experiments shown are performed with a serum calibration).

Table [SEQ Table * ARABIC]: Matrix Screen Results for Pair 1 (DAb 15 with CAb 8)

Matrix	Nominal Spike uIU/mL	[Nominal] uIU/mL	Signal (RLU)		Conc (uIU/mL)		
			Mean	CV %	Mean	CV %	% Recovery
Assay Buffer	25.00	25.00	379203	21.4	26.3	19.9	105
	10.00	10.00	110551	7.7	8.4	7.1	84
	5.00	5.00	72861	18.6	5.7	17.2	114
	2.50	2.50	28455	15.1	2.4	14.0	96
	1.00	1.00	11397	18.5	1.0	17.1	103
	0.00	0.00	1253	19.0	0.1	17.6	-
Depleted Serum	25.00	25.00	338106	20.5	23.6	18.8	95
	10.00	10.00	135347	15.3	10.1	14.2	101
	5.00	5.00	63772	21.7	5.1	20.1	101
	2.50	2.50	41221	15.7	3.4	14.6	135
	1.00	1.00	14536	14.5	1.3	13.4	129
	0.00	0.00	1130	9.7	0.1	8.9	-
Whole Blood	25.00	25.91	262651	15.7	18.7	14.5	72
	10.00	10.91	98346	19.8	7.5	18.4	69
	5.00	5.91	64521	26.4	5.1	24.4	86
	2.50	3.41	36334	12.7	3.0	11.7	88
	1.00	1.91	22945	20.9	1.4	71.7	71
	0.00	0.91	10952	4.4	0.9	14.4	-
Plasma	25.00	26.62	230962	11.5	16.6	10.6	62
(Directly Spiked)	10.00	11.62	110593	18.3	8.4	17.0	72
	5.00	6.62	55374	12.0	4.4	11.1	67
	2.50	4.12	45770	21.7	3.7	20.0	90
	1.00	2.62	28881	22.9	2.4	21.3	93
	0.00	1.62	18600	14.5	1.6	13.5	-

Assay Buffer Standard Curve Calibration Equation:
Conc = 10^(0.92509688*RLU - 3.74073340)

Table [SEQ Table * ARABIC]: Matrix Screen Results for Antibody Pair 2 (DAb 15 with CAb 12)

Matrix	Spiked [TSH] uIU/mL	Nominal [TSH] uIU/mL	Signal (RLU)		Conc. (uIU/mL)		
			Mean	CV %	Mean	CV %	% Recovery
Assay Buffer	25.00	25.00	161554	33.6	26.1	37.1	105
	10.00	10.00	56409	14.4	8.0	16.3	80
	5.00	5.00	45076	20.7	6.2	23.0	124
	2.50	2.50	19478	24.6	2.4	27.5	96
	1.00	1.00	9093	16.1	1.0	18.1	102
	0.00	0.00	1210	24.5	0.1	27.3	-
Depleted Serum	25.00	25.00	137312	11.0	21.7	12.4	87
	10.00	10.00	63247	20.7	9.1	23.5	91
	5.00	5.00	31663	8.0	4.2	9.0	83
	2.50	2.50	24755	24.3	3.2	27.3	126
	1.00	1.00	8506	17.7	0.9	19.8	95
	0.00	0.00	753	15.9	0.1	17.9	-
Whole Blood	25.00	25.72	152674	7.0	24.4	7.9	95
	10.00	10.72	60404	23.0	8.6	25.9	80
	5.00	5.72	40807	14.0	5.5	15.8	97
	2.50	3.22	25763	11.6	3.3	13.1	102
	1.00	1.72	13624	15.3	1.6	17.2	94
	0.00	0.72	6668	8.3	0.7	9.2	-
Plasma	25.00	25.86	170464	12.5	27.6	13.9	107
(Directly Spiked)	10.00	10.86	62524	23.0	9.0	26.0	82
	5.00	5.86	36872	12.2	4.9	13.7	84
	2.50	3.36	29001	13.4	3.8	15.0	112
	1.00	1.86	19156	16.3	2.1	30.5	115
	0.00	0.86	7802	24.6	0.9	27.5	-

Assay Buffer Standard Curve Calibration Equation:
Conc = 10^(1.12533965*RLU - 4.44650185)

Figure [SEQ Figure * ARABIC]: Matrix Screen, Antibody Pair 1

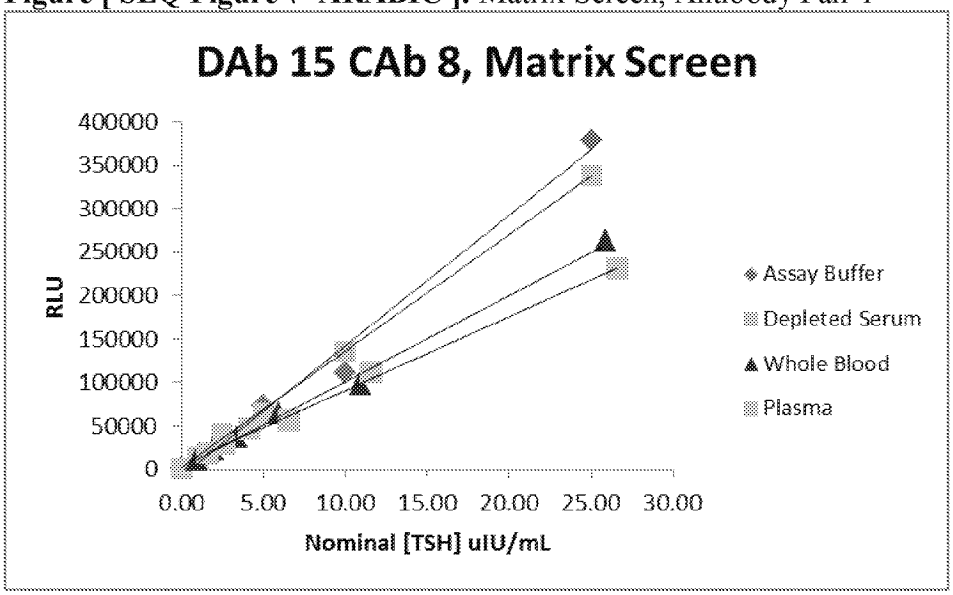


Figure [SEQ Figure * ARABIC]: Matrix Screen, Antibody Pair 2

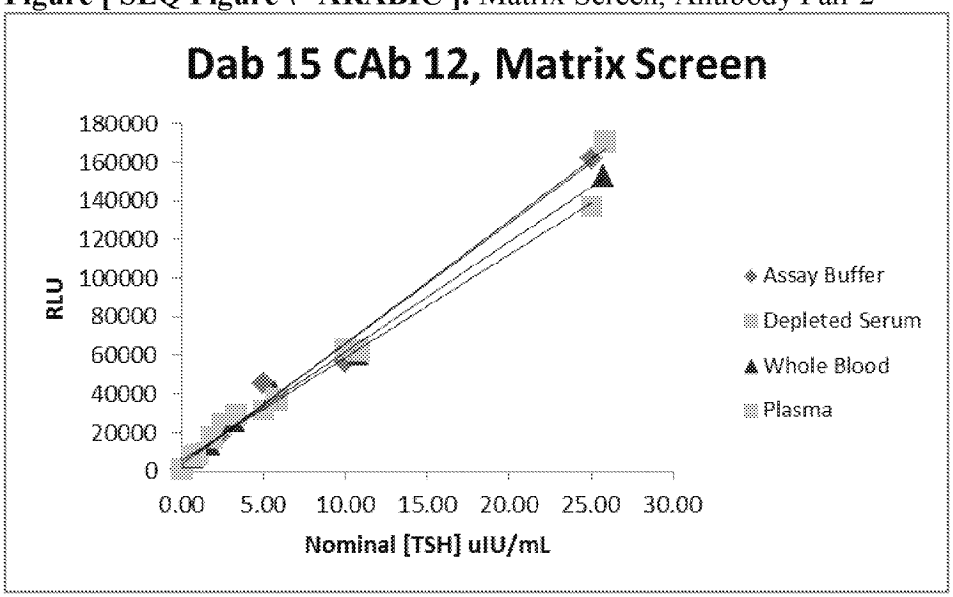
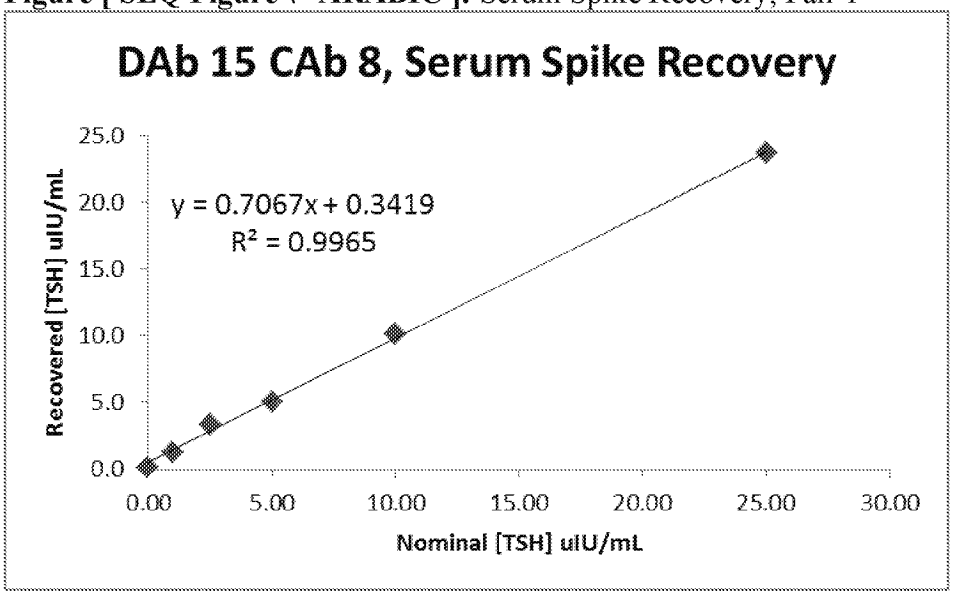
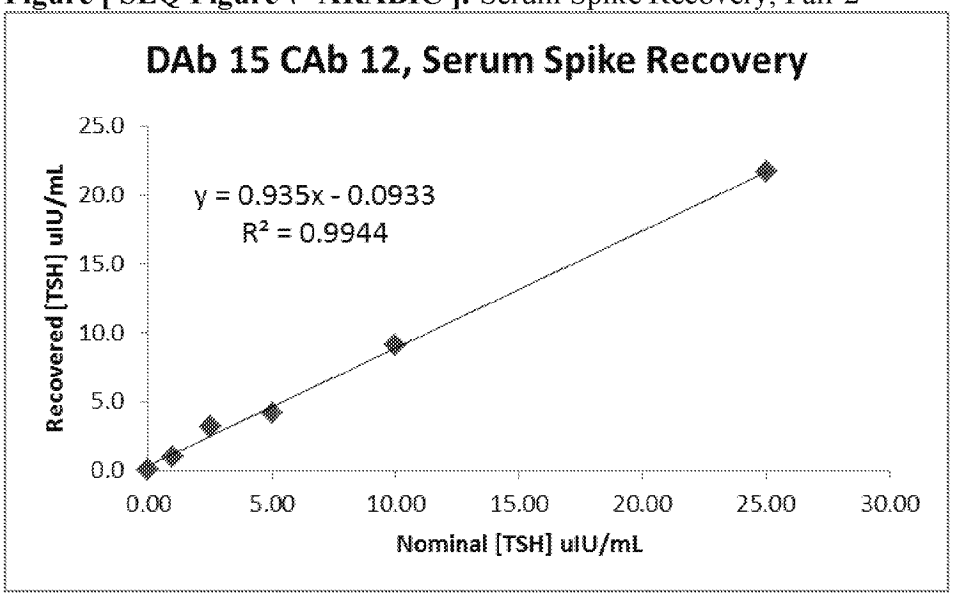


Figure [SEQ Figure * ARABIC]: Serum Spike Recovery, Pair 1



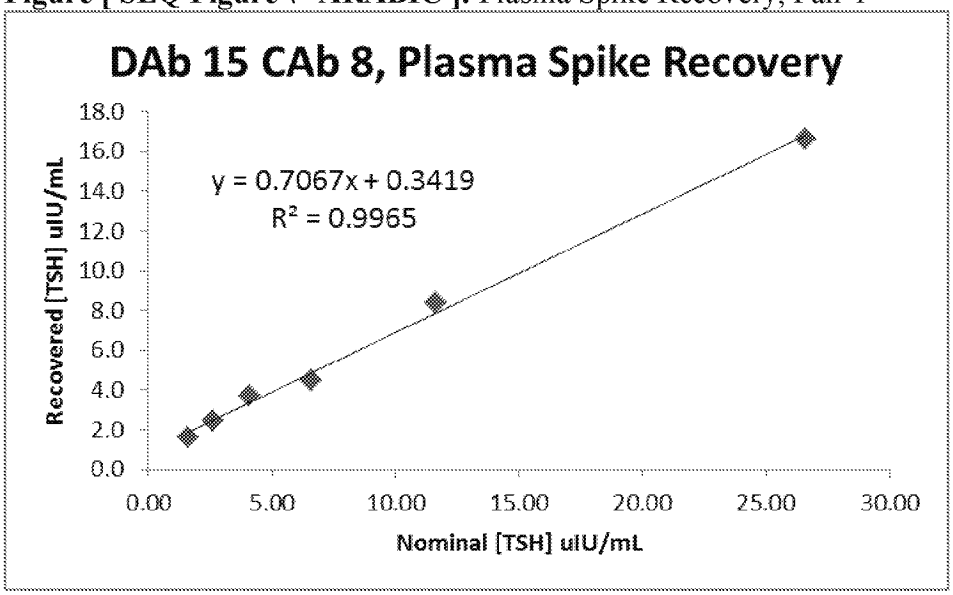
Spike recovery is calculated based on an assay buffer standard curve.

Figure [SEQ Figure * ARABIC]: Serum Spike Recovery, Pair 2



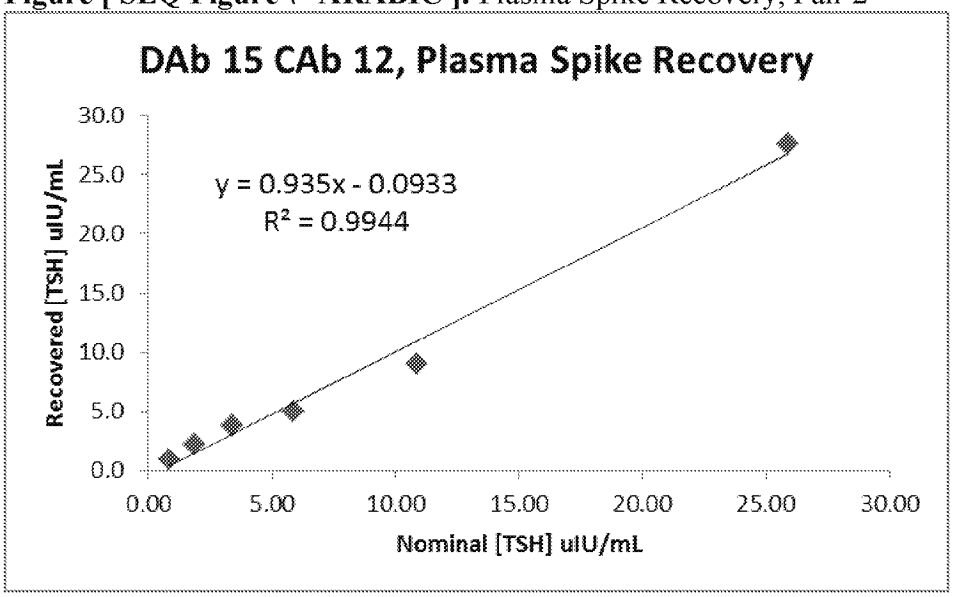
Spike recovery is calculated based on an assay buffer standard curve.

Figure [SEQ Figure * ARABIC]: Plasma Spike Recovery, Pair 1



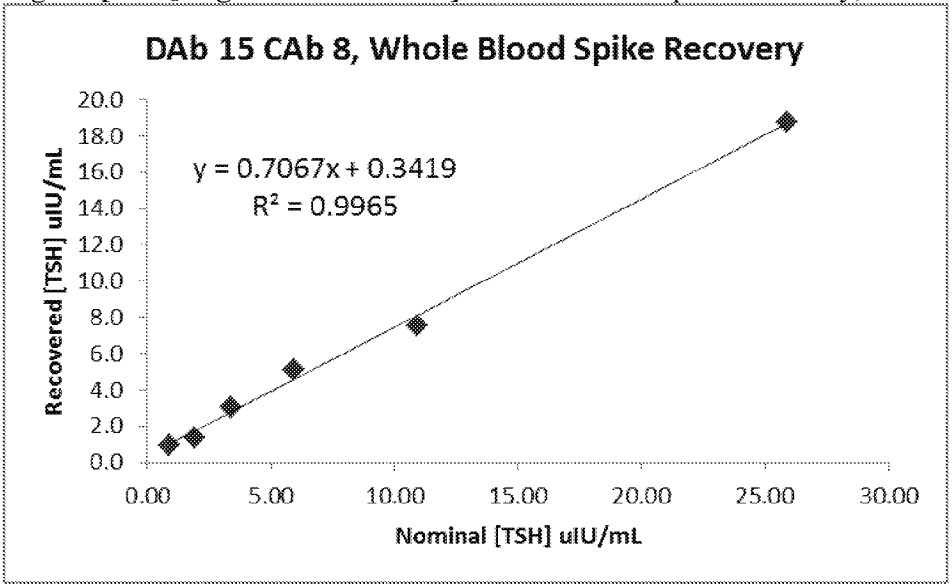
Spike recovery is calculated based on an assay buffer standard curve.

Figure [SEQ Figure * ARABIC]: Plasma Spike Recovery, Pair 2



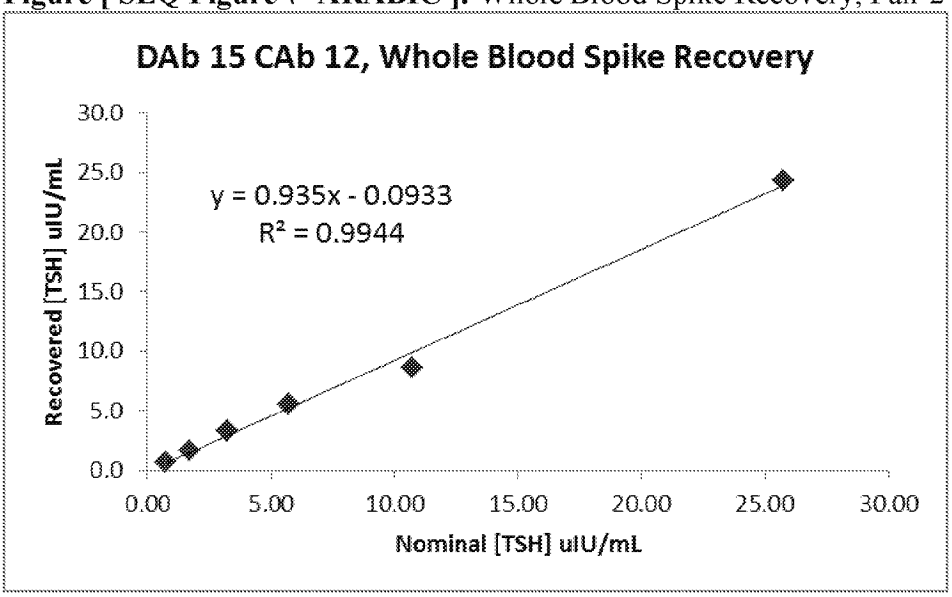
Spike recovery is calculated based on an assay buffer standard curve.

Figure [SEQ Figure * ARABIC]: Whole Blood Spike Recovery, Pair 1



Spike recovery is calculated based on an assay buffer standard curve.

Figure [SEQ Figure * ARABIC]: Whole Blood Spike Recovery, Pair 2



Spike recovery is calculated based on an assay buffer standard curve.

1.7 Finalization of Antibody Pair

Based on the results from the clinical sample training set, the matrix screen and the sensitivity of the calibrated assay, the final antibody pair was chosen as DAb 15 with CAb 12. Backup pairs are as follows:

Table [SEQ Table * ARABIC]: Finalized Ranking of Best Antibody Pairs

Rank	DAb#	CAb#
1	15	12
2	15	8
3	11	12
4	11	8
5	16	8
6	2	8

1.8 Epitope Mapping (MTP)

Epitope mapping was performed on a microtitre plate. The DAb was spiked with other anti-TSH unlabeled antibodies and testing for interference in the assay. The positive control was unlabeled Ab 15 spiked into the AP-labeled DAb15 to determine maximum competition for the DAb epitope. The testing was conducted with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. Since there are limited unique exposed epitopes on the TSH beta subunit, it is not surprising that many of the tested antibodies bind to the same or nearby epitopes as evidenced by interference in the assay.

Table [SEQ Table * ARABIC]: Epitope Mapping (MTP)

Antibody Number	Type	[TSH] uIU/mL	Mean RLU	CV %	% of CTL
Control	-	25	58957	2.2	-
		10	25059	1.0	-
		2.5	6178	0.5	-
		0	553	2.5	-
15	Mab	25	22088	3.8	37
(Positive Control)		10	8718	7.0	35
		2.5	2409	1.4	39
		0	553	3.5	100
1	Mab	25	56061	2.0	95
		10	24858	5.9	99
		2.5	5806	1.3	94
		0	600	10.7	109
2	Mab	25	32841	3.6	56
		10	13140	4.9	52
		2.5	3584	1.8	58

Antibody Number	Type	[TSH] uIU/mL	Mean RLU	CV %	% of CTL
		0	582	6.7	105
3	Mab	25	49512	11.2	84
		10	19919	3.3	79
		2.5	4998	2.1	81
		0	699	13.2	127
4	Mab	25	23576	0.2	40
		10	9263	2.3	37
		2.5	2415	0.1	39
		0	466	11.4	84
5	MAb	25	49219	2.2	83
		10	19166	1.7	76
		2.5	4996	2.1	81
		0	576	7.3	104
6	MAb	25	44872	1.0	76
		10	16864	1.8	67
		2.5	4772	0.1	77
		0	606	2.8	110
7	MAb	25	47024	2.2	80
		10	18330	3.4	73
		2.5	4463	4.5	72
		0	634	44.2	115
8	MAb	25	45187	2.4	77
		10	18717	1.6	75
		2.5	4483	1.3	73
		0	711	9.1	129
9	MAb	25	44541	0.3	76
		10	17257	1.5	69
		2.5	4418	0.4	72
		0	725	20.1	131
10	MAb	25	52967	2.7	90
		10	21173	1.9	84
		2.5	5563	3.1	90
		0	622	14.4	113
11	MAb	25	47806	2.5	81
		10	18691	2.6	75
		2.5	5113	6.1	83
		0	656	4.7	119
14	MAb	25	48066	0.6	82
		10	18877	0.8	75
		2.5	5184	4.3	84

Antibody Number	Type	[TSH] uIU/mL	Mean RLU	CV %	% of CTL
		0	563	2.0	102
16	MAb	25	21126	4.1	36
		10	8645	1.3	34
		2.5	2243	0.0	36
		0	531	1.1	96
17	PAb	25	36267	2.5	62
		10	14685	9.2	59
		2.5	4016	2.0	65
		0	616	20.5	111
18	MAb	25	37328	1.6	63
		10	14707	1.4	59
		2.5	4208	3.6	68
		0	551	17.3	100
19	MAb	25	39323	3.7	67
		10	15731	3.2	63
		2.5	4275	8.5	69
		0	567	1.0	103

1.9 Whole Blood Screen

To verify the normal range in whole blood, 11 samples were screened with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. The samples were screened on 3 different days with different reagent lots, so the results are calibrated on the appropriate standard curve generated on the same day. These results corresponded with the expected normal range in serum for adults of 0.3 – 5 uIU/mL.

Table [SEQ Table * ARABIC]: Whole Blood Screen

Sample	Date Tested	Sample ID	Signal, RLU		Conc. uIU/mL	
			Mean	CV %	Mean	CV %
1	03/02/11	W070511111027	16336	11.4	2.17	7.6
2	03/02/11	W070511111020	5067	24.2	0.62	1.0
3	03/02/11	W070511200333	17772	13.1	2.37	10.0
4	03/02/11	W070511100274	18052	20.4	2.41	13.3
5	03/15/11	W070511111276	10369	8.9	1.18	8.7
6	03/15/11	W070511111277	8986	4.2	1.01	0.6
7	03/15/11	W070511111280	15614	12.5	1.87	15.5
8	03/15/11	W070511111281	24815	13.9	3.16	19.1
9	03/30/11	W070511100454	24788	16.1	2.07	16.4

10	03/30/11	W070511111745	21157	21.4	1.77	15.2
11	03/30/11	W070511111747	17900	12.9	1.50	11.7

1.10 Plasma Screen

To verify the normal range in plasma, 10 samples were screened with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL and a 1:5 sample dilution. These results corresponded with the expected normal range in serum for adults of 0.3 – 5 uIU/mL.

Table [SEQ Table * ARABIC]: Plasma Screen

Sample	Date Tested	Sample ID	Signal, RLU		Conc. uIU/mL	
			Mean	CV %	Mean	CV %
1	03/30/11	W070510113216	17471	10.8	1.46	12.0
2	03/30/11	W070510113218	38871	21.0	3.22	20.2
3	03/30/11	W070510113714	18755	14.6	1.57	2.9
4	03/30/11	W070511111027	58207	17.4	4.77	9.2
5	03/30/11	W070511111020	14241	13.7	1.18	11.8
6	03/30/11	W070511200333	32916	15.9	2.74	11.2
7	03/30/11	W070511100274	47225	10.8	3.89	2.4
8	03/30/11	W070511100454	43156	18.3	3.42	18.5
9	03/30/11	W070511111745	35374	9.8	2.94	9.6
10	03/30/11	W070511111747	30106	12.0	2.51	10.3

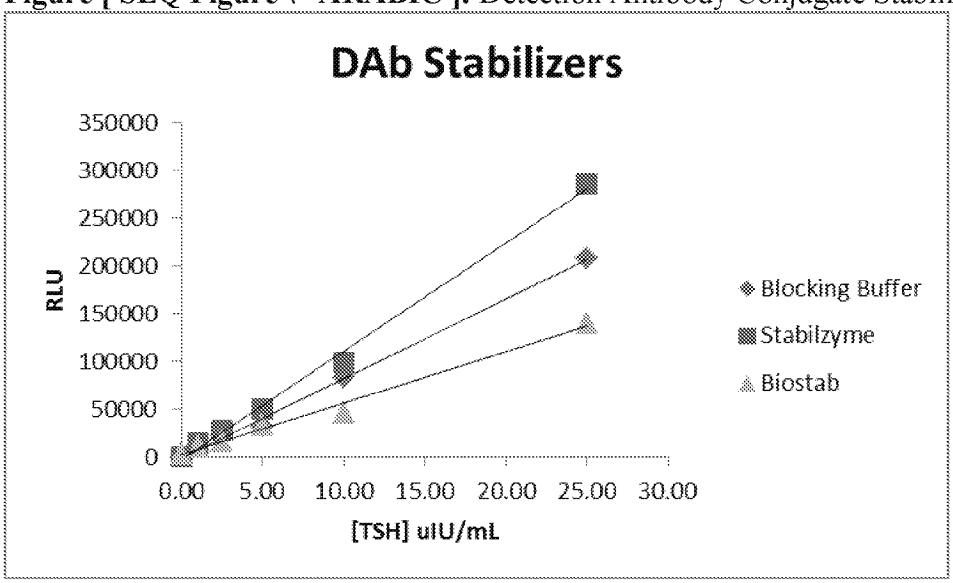
1.11 Detection Antibody Conjugate Stabilizers

Two different commercial alkaline phosphatase stabilizing buffers were tested with assay conditions of 100 ng/mL DAb, 10 ug/mL CAb and a 1:5 sample dilution of serum calibrators. StabilZyme produced the best signal to background (S/B) and was chosen as the DAb diluent.

Table [SEQ Table * ARABIC]: Detection Antibody Conjugate Stabilizers

[TSH] uIU/mL	Blocking Buffer		Stabilzyme		Biostab	
	Mean	CV %	Mean	CV %	Mean	CV %
25.00	208055	12.0	285880	3.0	139669	18.3
10.00	82539	7.2	99426	21.8	45774	4.1
5.00	31751	7.9	50184	14.3	32448	10.5
2.50	23103	4.6	28053	10.5	15849	9.2
1.00	11370	25.4	14077	18.2	11010	22.8
0.00	714	4.7	572	16.5	2335	7.9
S/B	291		500		60	

Figure [SEQ Figure * ARABIC]: Detection Antibody Conjugate Stabilizers



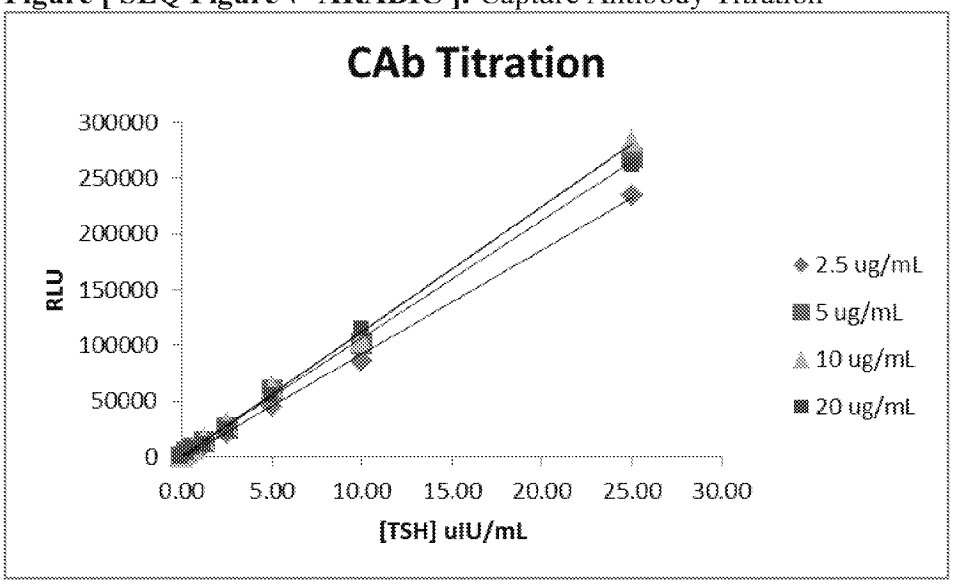
1.12 Capture Antibody Titration

The biotin conjugated capture antibody was titrated at 4 levels to determine the ideal coating concentration, with a DAb concentration of 100 ng/mL in StabilZyme and a 1:5 sample dilution of serum calibrators. A concentration of 5 ug/mL was optimal for modulation across the range and at the low end.

Table [SEQ Table * ARABIC]: Capture Antibody Titration

	2.5 ug/mL		5 ug/mL		10 ug/mL		20 ug/mL	
[TSH] uIU/mL	**Mean**	**CV %**	**Mean**	**CV %**	**Mean**	**CV %**	**Mean**	**CV %**
25.00	234776	13.8	269155	5.9	283115	7.7	261580	9.6
10.00	85747	24.5	101701	13.4	104177	17.4	115029	6.5
5.00	45417	6.1	60454	5.6	61977	7.8	55787	4.5
2.50	21749	28.3	26375	17.5	29606	16.7	24895	10.4
1.25	10380	15.4	13305	9.8	15556	7.2	11743	10.0
0.63	6479	11.8	7526	13.9	7191	14.6	7607	13.4
0.31	3228	8.4	4130	16.2	4005	10.8	3908	6.8
0.00	671	18.1	552	9.8	596	19.7	542	15.0
S/B	350		488		475		483	
S/B @ 0.31 uIU/mL	5		7		7		7	

Figure [SEQ Figure * ARABIC]: Capture Antibody Titration



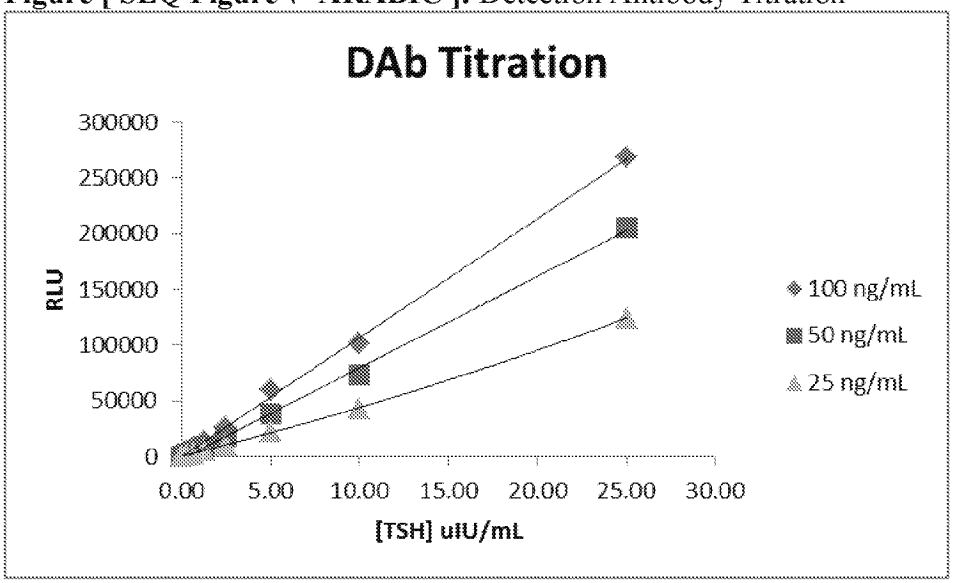
1.13 Detection Antibody Titration

The detection antibody was titrated in StabilZyme at 3 levels, with CAb at 5 ug/mL and a 1:5 sample dilution of serum calibrators, to determine the optimal working concentration. The original concentration of 100 ng/mL provided the best modulation across the range, and in particular at the low end of the assay where sensitivity is desired. Background did not decrease significantly as the detection antibody was titrated lower, therefore 100 ng/mL was chosen as the final concentration.

Table [SEQ Table * ARABIC]: Detection Antibody Titration

	100 ng/mL		50 ng/mL		25 ng/mL	
[TSH] uIU/mL	**Mean**	**CV %**	**Mean**	**CV %**	**Mean**	**CV %**
25.00	269155	5.9	205374	5.6	124340	8.8
10.00	101701	13.4	73680	15.0	43352	14.5
5.00	60454	5.6	38522	8.9	22497	12.6
2.50	26375	17.5	17728	18.7	10593	16.7
1.25	13305	9.8	9427	5.6	6028	10.0
0.63	7526	13.9	4445	13.7	2695	12.3
0.31	4130	16.2	2459	15.5	1500	13.4
0.00	552	9.8	458	23.5	335	39.4
S/B	488		449		371	
S/B @ 0.31 uIU/mL	7		5		4	

Figure [SEQ Figure * ARABIC]: Detection Antibody Titration



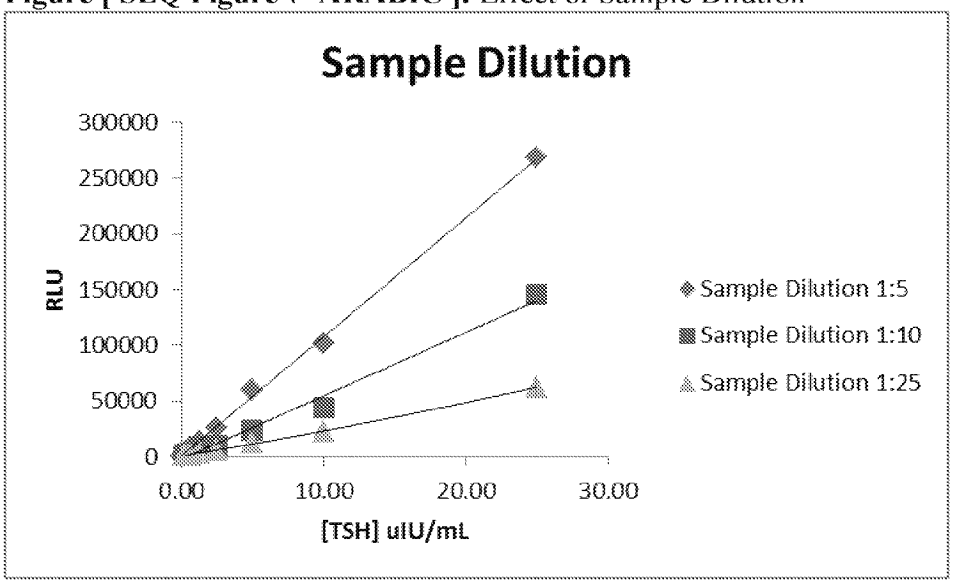
1.14 Effect of Sample Dilution

The effect of increasing sample dilutions was tested, with 1:5, 1:10 and 1:25 dilutions. Assay conditions were DAb 100 ng/mL in StabilZyme and 5 ug/mL CAb. The 1:5 sample dilution provides a very linear assay with good sensitivity. At 1:10 the signal to background is significantly lower and the sensitivity is decreased. At 1:25 the assay modulation is not sufficient to ensure the required sensitivity of 0.1 uIU/mL.

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

	Sample Dilution 1:5		Sample Dilution 1:10		Sample Dilution 1:25	
[TSH] uIU/mL	**Mean RLU**	**CV %**	**Mean RLU**	**CV %**	**Mean RLU**	**CV %**
25.00	269155	5.9	145239	8.9	62685	13.3
10.00	101701	13.4	44513	13.9	22456	16.1
5.00	60454	5.6	23483	7.4	12730	10.2
2.50	26375	17.5	10591	17.1	6170	24.8
1.25	13305	9.8	8653	11.1	3094	12.7
0.63	7526	13.9	3936	15.0	1694	38.5
0.31	4130	16.2	2585	14.5	1360	9.2
0.16	2654	9.8	1576	11.4	975	8.2
0.00	552	9.8	528	6.4	499	5.2
S/B	488		275		126	
S/B @ 0.16 uIU/mL	5		3		2	

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



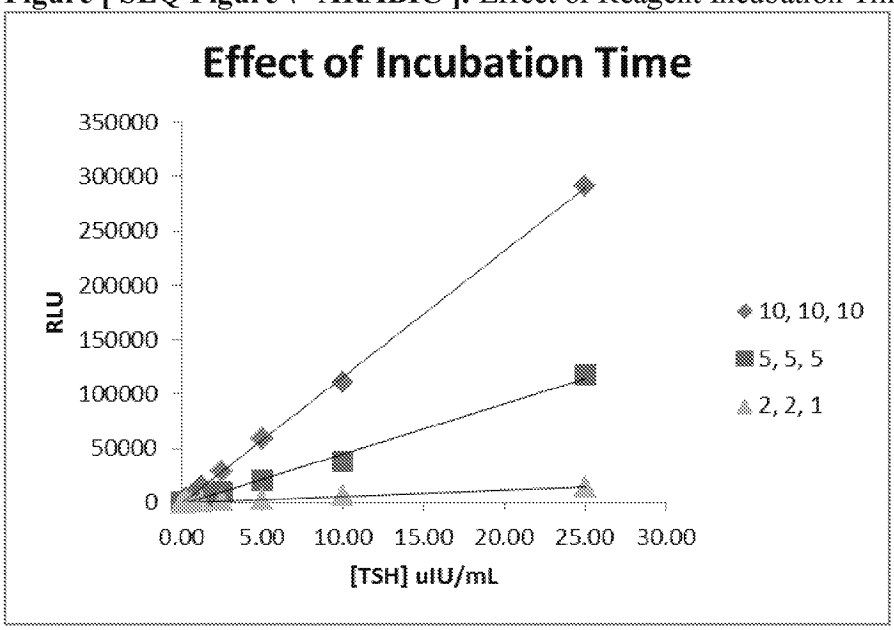
1.15 Effect of Reagent Incubation Time

The effect of shorter reagent incubation times was tested with 10-10-10 minute, 5-5-5 minute and 2-2-1 minute sample, detection conjugate, and substrate incubations respectively. Assay conditions were DAb 100 ng/mL in StabilZyme, 5 ug/mL CAb, and 1:5 sample dilution. Shortening the incubation times to 5 minutes significantly decreased the signal to background ratio (S/B) and at 2-2-1 minute incubations the modulation at the low end of the assay was lost. A 10-10-10 assay format is optimal.

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

[TSH] uIU/mL	10, 10, 10		5, 5, 5		2, 2, 1	
	Mean	CV %	Mean	CV %	Mean	CV %
25.00	291255	6.9	117314	7.7	14782	10.6
10.00	111214	13.8	37456	14.6	6152	21.3
5.00	59055	4.2	20370	10.7	3195	14.5
2.50	29131	20.5	10377	20.7	2282	45.1
1.25	15595	9.9	5330	22.9	885	9.6
0.63	7204	11.4	2669	11.8	615	5.0
0.31	4591	14.8	1482	18.8	363	11.4
0.16	2654	9.8	1060	20.3	280	6.4
0.00	552	9.8	389	18.6	189	22.6
S/B	528		301		78	
S/B @ 0.16	5		3		1	

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



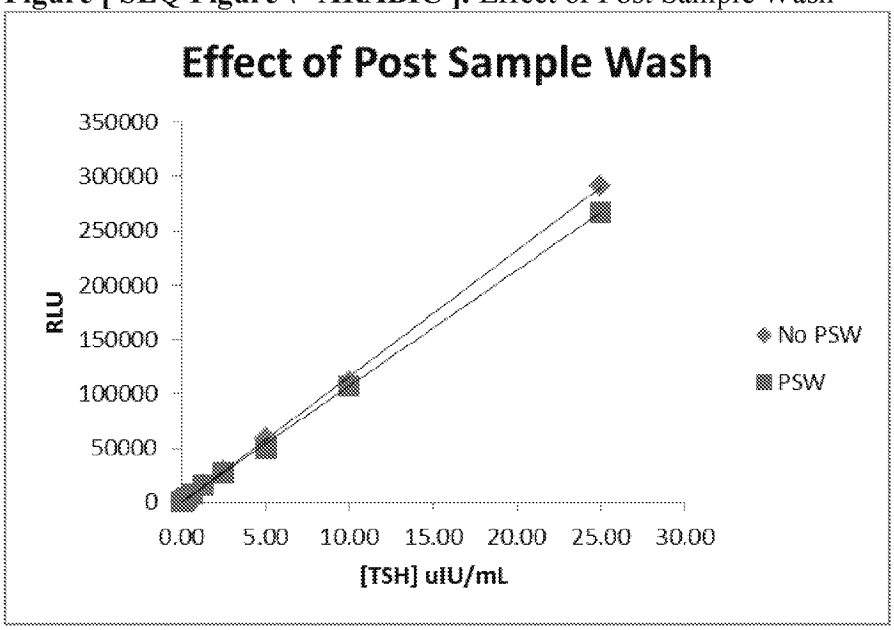
1.16 Effect of Post Sample Wash

The effect of a post sample wash was tested with DAb at 100 ng/mL in StabilZyme, CAb 5 ug/mL and a sample dilution of 1:5 with serum calibrators. Adding a post sample washing step slightly reduced modulation across the range, and in particular at the low end. No post sample wash is the optimal condition for this assay.

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

[TSH] uIU/mL	No Post Sample Wash		Post Sample Wash	
	Mean RLU	CV %	Mean RLU	CV %
25.00	291255	6.9	266983	5.9
10.00	111214	13.8	107358	4.1
5.00	59055	4.2	49643	9.9
2.50	29131	20.5	27412	13.6
1.25	15595	9.9	15615	11.7
0.63	7204	11.4	8244	12.8
0.31	4591	14.8	4005	6.4
0.16	2654	9.8	2531	4.9
0.00	552	9.8	597	12.5
S/B	528		447	
S/B @ 0.16 uIU/mL	5		4	

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



1.17 Determination of Expected LLOQ and ULOQ

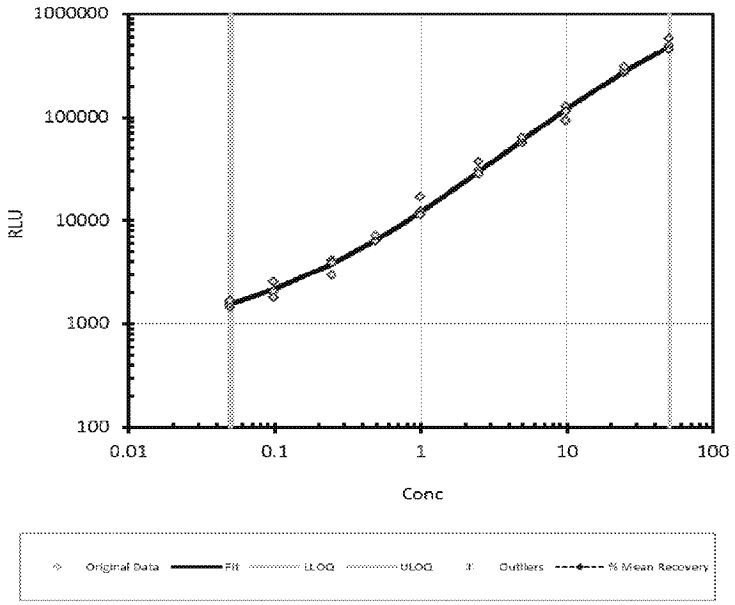
A lot of reagents was produced and a serum calibration was performed on the final assay conditions of 100 ng/mL DAb in StabilZyme, 5 ug/mL CAb, and a 1:5 sample dilution with 3 cartridges per point. The LLOQ was 0.05 uIU/mL and the ULOQ was 50 uIU/mL.

Table [SEQ Table * ARABIC]: Serum Standard Curve for Determination of LLOQ and ULOQ

[TSH] uIU/mL	Signal (RLU)		Back-Calculated Conc. (uIU/mL)		
	Mean	CV %	Mean	CV %	% Recovery
50.00	511707	14.0	51.28	10.5	103
25.00	284858	8.7	25.32	7.9	101
10.00	111303	14.2	9.09	15.2	91
5.00	60195	5.3	4.93	5.4	99
2.50	32071	15.2	2.67	14.5	107
1.00	13532	20.7	1.12	23.6	112
0.50	6628	6.6	0.51	7.6	101
0.25	3669	16.5	0.23	24.9	93
0.10	2138	16.1	0.10	33.2	96
0.05	1577	10.7	0.06	8.3	111
0.00	592	6.6	-	-	-

Conc = 3.650 * (((6.525 - 2.765) / (log10(RLU) - 2.765)) - 1) ^ (1 / -0.476)
Signal Min = 1439, Signal Max = 534108

Figure [SEQ Figure * ARABIC]: Serum Standard Curve for Determination of LLOQ and ULOQ



1.18 Hematocrit Effect

The hematocrit effect was determined with 3 samples of whole blood spiked at three levels and at 0. The serum calibration shown in section 1.16 was applied. The spiked whole blood samples were measured, then the remaining spiked whole blood was centrifuged and the resulting plasma was measured on the Theranos System. The spiked TSH and endogenous TSH was found to fully concentrate into the plasma prepared from spiked whole blood. The recovery of TSH in plasma was uniform across the range and for 3 different whole blood samples.

Table [SEQ Table * ARABIC]: Hematocrit Effect, Signal (RLU)

Sample	[TSH] uIU/mL Spiked	Whole Blood		Plasma	
		Mean	CV %	Mean	CV %
1	18.00	210901	19.7	362402	12.0
	9.00	104751	13.8	212186	16.3
	3.00	57917	19.7	102487	12.5
	0.00	24788	16.1	43156	18.3
2	18.00	209171	13.6	398353	14.1
	9.00	105241	12.3	178598	13.4
	3.00	44513	19.0	88297	12.1
	0.00	21157	21.4	35374	9.8
3	18.00	207921	13.2	383076	13.1
	9.00	99546	11.2	207095	16.8
	3.00	46689	18.1	81643	13.6
	0.00	17900	12.9	30106	12.0

Table [SEQ Table * ARABIC]: Hematocrit Effect, Concentration (uIU/mL)

Sample	[TSH] uIU/mL Spiked	Whole Blood				Plasma	
		Nominal	Mean	CV %	% Recovery	Mean	CV %
1	18.00	20.07	16.98	26.2	85	34.14	6.9
	9.00	11.07	8.46	14.1	76	18.07	17.6
	3.00	5.07	5.00	21.5	98	8.36	14.0
	0.00	2.07	2.07	16.4		3.42	18.5
2	18.00	19.77	17.77	14.2	90	37.30	7.4
	9.00	10.77	8.58	0.1	80	14.93	14.7
	3.00	4.77	3.68	20.4	77	7.53	8.3
	0.00	1.77	1.77	15.2		2.94	9.6
3	18.00	19.50	17.65	11.5	90	35.01	9.3
	9.00	10.50	8.11	10.1	77	17.59	6.5
	3.00	4.50	3.85	11.5	86	6.66	12.7
	0.00	1.50	1.50	11.7		2.51	10.3

Figure [SEQ Figure * ARABIC]: Whole Blood Spike Recovery, 3 Samples

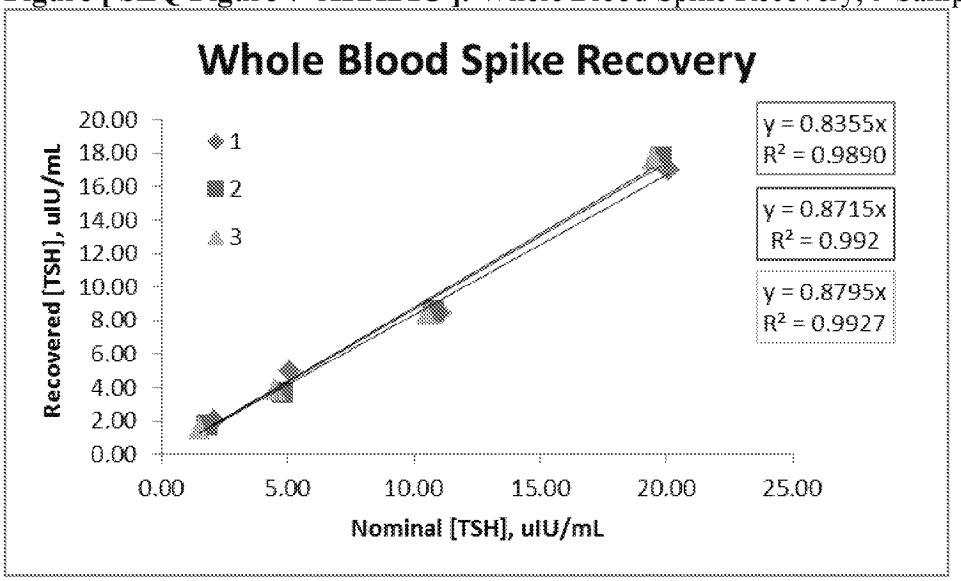
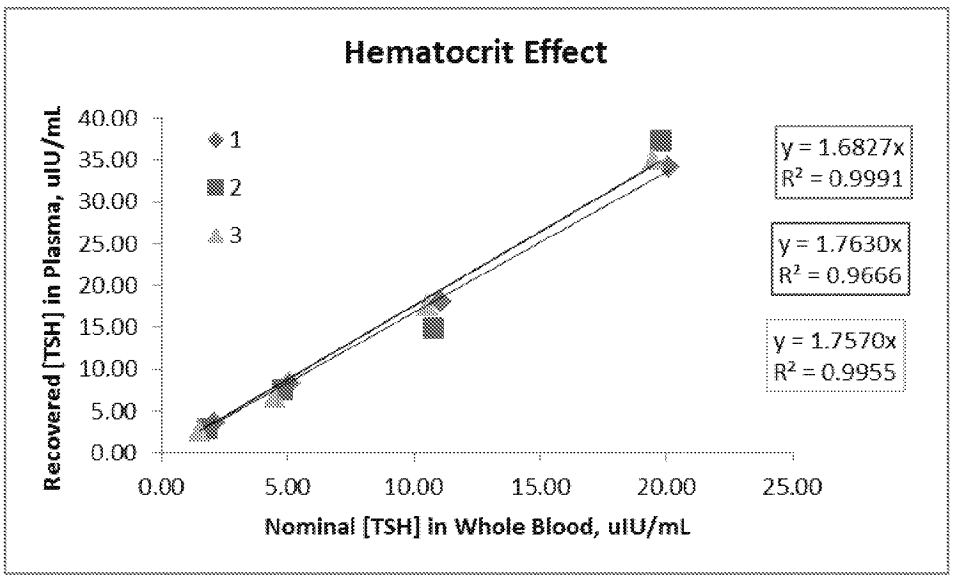


Figure [SEQ Figure * ARABIC]: Hematocrit Effect, Recovery of a Whole Blood Spike in the Plasma



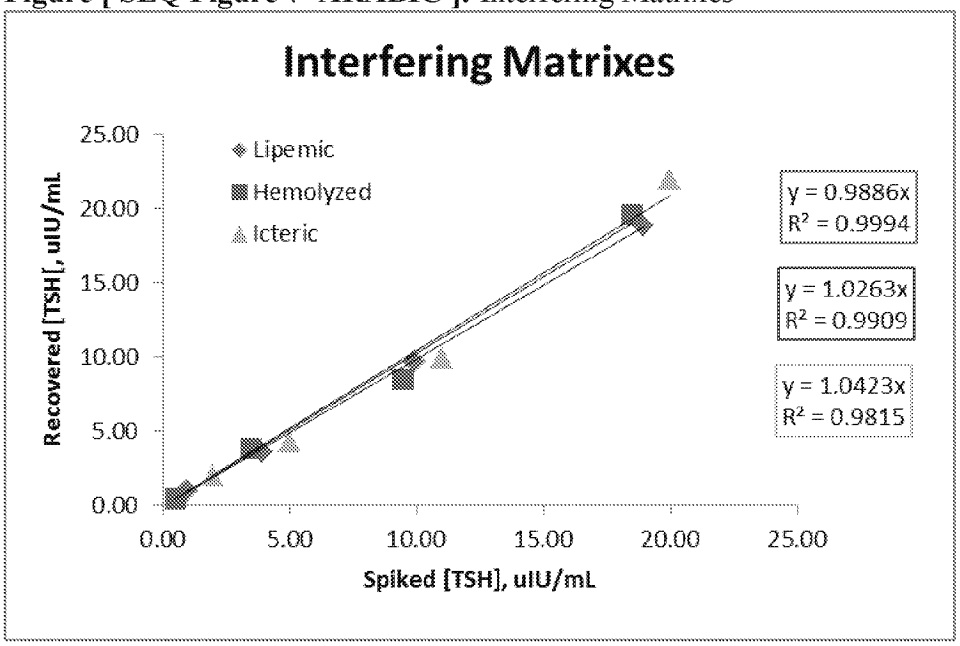
1.19 Interfering Matrixes

Hemolyzed, icteric, and lipemic serum samples were obtained from ProMedDx. The recovery of TSH spiked into these potentially interfering matrixes was evaluated on the Theranos System. The serum calibration shown in section 1.16 was applied. The assay did not show any interference from hemolysed, icteric, or lipemic samples, spike recovery was within 15% of nominal.

Table [SEQ Table * ARABIC]: Interfering Matrixes

Sample Type	[TSH] uIU/mL		Signal (RLU)		Conc (uIU/mL)		
	Spiked	Nominal	Mean	CV %	Mean	CV %	% Recovery
Hemolyzed	18.00	18.49	226260	6.3	19.54	4.2	106
	9.00	9.49	104433	16.7	8.52	18.2	90
	3.00	3.49	46691	5.3	3.85	4.5	111
	0.00	0.49	6397	14.4	0.49	18.0	-
Icteric	18.00	19.99	251772	11.6	21.93	14.0	110
	9.00	10.99	121435	16.3	9.94	11.0	90
	3.00	4.99	51630	17.2	4.25	15.9	85
	0.00	1.99	23814	12.5	1.99	13.4	-
Lipemic	18.00	18.93	221011	4.2	18.85	4.6	100
	9.00	9.93	118224	7.6	9.66	6.0	97
	3.00	3.93	43925	10.6	3.63	8.1	92
	0.00	0.93	11356	18.9	0.93	22.2	-

Figure [SEQ Figure * ARABIC]: Interfering Matrixes



1.20 Stability

TBD