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Anti-TPO Ab Assay Development Report

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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"]

An enzyme linked immunosorbent assay (ELISA) was developed for the quantitative detection of antibodies to thyroid peroxidase (TPO). TPO is a 105kD membrane bound enzyme important for the production of thyroid hormones. Autoantibodies to TPO interfere with T3 and T4 production, which leads to autoimmune disorders such as Hashimoto's and Grave's disease. This report describes the assay development and performance of the anti-TPO antibody assay as aid to diagnosing Hashimoto's and Grave's disease.

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

The following assays were used as predicament methods.

Anti-TPO Ab, Siemens, Immulite 2000, Cat. L2KT02
Anti-TPO ELISA, DRG, Cat. EIA-4114

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

The anti-TPO ELISA was developed using biotinylated TPO on an ultravidin (UA) surface as the capture surface. Anti-TPO antibodies in serum or plasma bind specifically to the TPO for 5 minutes followed by a wash cycle. After washing, the anti-TPO antibodies were detected using an AP labeled mouse monoclonal antibody to human IgG. After incubation with the detector antibody for 5 minutes, another wash cycle was performed, and the alkaline phosphatase substrate added. The resulting chemiluminescence was read in relative light units (RLU) on the Theranos system.

Table [SEQ Table * ARABIC]: Materials

Item	Supplier	Catalog #
TPO (recombinant)	Fitzgerald	30-AT61
Anti-TPO WHO Standard	NIBSC	66/387
Mouse Anti-human IgG clone JDC-10	Southern Biotech	9040-01
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13-10
Biotin Labeling Kit (NH)	Dojindo	LK03-10
Phospha Glo Substrate	KPL	55-60-04
Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G
Conjugate Diluent (3% BSA/0.1 mM Zn2+/5mM Mg2+)	Theranos	66/387
Tris Buffered Saline	Sigma	T6664-10PAK
Theranos Cartridge	Theranos	
Theranos System	Theranos	

1.1.3 Labeling of Detector Antibody

The mouse anti-human IgG clone JDC-10 was labeled with alkaline phosphatase according to kit instructions (Dojindo, LK13-10).

1.1.4 Biotin TPO

The biotinylation of TPO was done at room temperature using the Dojindo kit (Dojindo, LK03-10).

1.1.5 Preparation of Assay Buffer

The assay buffer was prepared by dissolving 1 packet of TBS into water and adding 10 mL of 10% azide, and 30 g of BSA to a final volume of 1000mL. The final composition of the assay buffer is 3% BSA, 50mM Tris, 138mM NaCl, 2.7mM KCl pH 8.0 in water. The assay buffer was filtered before use.

1.1.6 Preparation of Conjugate Diluent

The conjugate diluent was prepared by adding $ZnCl_2$ and $MgCl_2$ to the assay buffer to final concentrations of 0.1mM $^{Zn2+}$ and 5mM Mg^{2+}.

2. ASSAY DEVELOPMENT

2.1 Capture Surface

The assay capture surface was determined by comparing TPO to biotin TPO on the Theranos system. Biotin TPO at 1 ug/mL was compared to TPO at 1 ug/mL, 5 ug/mL, and 10 ug/mL. The sample dilution was 10x with 10-10-10 incubation times and no post sample wash. The detector concentration was 100 ng/mL. The calibrator was from Calbiotech and had no assigned units. Biotin TPO was selected as the final capture surface since it gave better modulations and precision compared to TPO.

Table 2: TPO and Biotin TPO

Capture	Dilution	AVG RLU	CV	Modulation
Biotin-TPO @ 1 ug/mL	0.500	352923	18%	639.3
Biotin-TPO @ 1 ug/mL	0.125	89174	8%	161.5
Biotin-TPO @ 1 ug/mL	0.031	20861	9%	37.8
Biotin-TPO @ 1 ug/mL	0.008	9523	23%	17.2
Biotin-TPO @ 1 ug/mL	0.002	3184	7%	5.8
Biotin-TPO @ 1 ug/mL	0.000	552	24%	1.0
TPO @ 1 ug/mL	0.500	31761	29%	124.4
TPO @ 1 ug/mL	0.125	7297	14%	28.6
TPO @ 1 ug/mL	0.031	1548	27%	6.1
TPO @ 1 ug/mL	0.008	812	25%	3.2
TPO @ 1 ug/mL	0.002	386	22%	1.5
TPO @ 1 ug/mL	0.000	255	3%	1.0
TPO @ 5 ug/mL	0.500	247131	28%	530.9
TPO @ 5 ug/mL	0.125	60164	27%	129.2
TPO @ 5 ug/mL	0.031	13856	3%	29.8
TPO @ 5 ug/mL	0.008	4598	28%	9.9
TPO @ 5 ug/mL	0.002	1067	42%	2.3
TPO @ 5 ug/mL	0.000	465	17%	1.0
TPO @ 10 ug/mL	0.500	405183	45%	524.9
TPO @ 10 ug/mL	0.125	77018	2%	99.8
TPO @ 10 ug/mL	0.031	19945	13%	25.8
TPO @ 10 ug/mL	0.008	5798	20%	7.5
TPO @ 10 ug/mL	0.002	1514	17%	2.0
TPO @ 10 ug/mL	0.000	772	8%	1.0

2.2 Effect of Post Sample Wash

The effect of post sample wash was tested by comparing different 10x sample dilution protocols. The experiment was done using unconjugated TPO at 5 ug/mL as the capture surface since there was no biotin TPO available at the time. The AP labeled Southern Biotech clone JDC-10 detector was used at 100 ng/mL. The modulations were better with a post sample wash.

Table 3: Effect of Post Sample Wash

Protocol	Dilution	AVG RLU	CV	Modulation
Generic2_10x_10-10-10	0.500	308328	26%	485.3
Generic2_10x_10-10-10	0.125	60955	18%	95.9
Generic2_10x_10-10-10	0.031	15768	19%	24.8
Generic2_10x_10-10-10	0.008	4835	11%	7.6
Generic2_10x_10-10-10	0.002	1229	17%	1.9
Generic2_10x_10-10-10	0.000	635	6%	1.0
Generic2_10x_PSW	0.500	184837	69%	569.5
Generic2_10x_PSW	0.125	44950	13%	138.5
Generic2_10x_PSW	0.031	13201	23%	40.7
Generic2_10x_PSW	0.008	3779	19%	11.6
Generic2_10x_PSW	0.002	1387	8%	4.3
Generic2_10x_PSW	0.000	325	21%	1.0

2.3 Capture Surface Titration

The capture titration experiment was performed using biotin TPO as the final capture surface and a post sample wash protocol. Tips were coated with biotin TPO at 10 ug/mL, 5 ug/mL, 1 ug/mL, and 0.5 ug/mL in 3% BSA blocking buffer. The sample dilution was 10x with incubations times of 10-10-10 and post sample wash. The AP conjugated Southern Biotech detector was used at 100 ng/mL. Good precision and sensitivity was achieved at 1 ug/mL biotin TPO.

Table 4: Capture Surface Titration

Biotin TPO	Dilution	AVG RLU	CV	Modulation
10 ug/mL	0.500	1752952	5%	1282
10 ug/mL	0.125	767211	11%	561
10 ug/mL	0.031	309117	11%	226
10 ug/mL	0.008	90385	15%	66
10 ug/mL	0.002	22403	18%	16
10 ug/mL	0.000	1368	19%	1
5 ug/mL	0.500	1660855	26%	1155
5 ug/mL	0.125	765450	10%	532
5 ug/mL	0.031	289508	26%	201
5 ug/mL	0.008	85554	13%	60
5 ug/mL	0.002	25953	6%	18
5 ug/mL	0.000	1438	25%	1
1 ug/mL	0.500	1276912	13%	1305
1 ug/mL	0.125	428495	11%	438
1 ug/mL	0.031	145535	23%	149
1 ug/mL	0.008	44518	7%	45
1 ug/mL	0.002	13812	13%	14
1 ug/mL	0.000	979	16%	1
0.5 ug/mL	0.500	973172	17%	1315
0.5 ug/mL	0.125	306810	20%	415
0.5 ug/mL	0.031	109622	16%	148
0.5 ug/mL	0.008	27215	14%	37
0.5 ug/mL	0.002	8996	12%	12
0.5 ug/mL	0.000	740	12%	1

2.4 Detector Antibodies

The best antibody for detection was determined by testing 3 detection antibodies from 3 different vendors using a 10x sample dilution and 10-10-10 protocol with a post sample wash. All the detectors were mouse monoclonals against human IgG. The Southern Biotech detector gave the best modulations and also the lowest background.

Table 5: Detector Antibodies

Detector	Dilution	AVG RLU	CV	Modulation
Southern Biotech, clone JDC-10, Cat. 9040-01	0.500	311023	19%	1086.2
Southern Biotech, clone JDC-10, Cat. 9040-01	0.125	31601	10%	110.4
Southern Biotech, clone JDC-10, Cat. 9040-01	0.031	8283	26%	28.9
Southern Biotech, clone JDC-10, Cat. 9040-01	0.008	2814	10%	9.8
Southern Biotech, clone JDC-10, Cat. 9040-01	0.002	1243	10%	4.3
Southern Biotech, clone JDC-10, Cat. 9040-01	0.000	286	27%	1.0
Novus, clone 2CH, NB100-2046	0.500	6920	32%	17.8
Novus, clone 2CH, NB100-2046	0.125	1726	17%	4.4
Novus, clone 2CH, NB100-2046	0.031	688	22%	1.8
Novus, clone 2CH, NB100-2046	0.008	485	17%	1.2
Novus, clone 2CH, NB100-2046	0.002	323	7%	0.8
Novus, clone 2CH, NB100-2046	0.000	389	26%	1.0
US Biological, clone 8L354, I1904-75W	0.500	4445	42%	10.4
US Biological, clone 8L354, I1904-75W	0.125	1741	16%	4.1
US Biological, clone 8L354, I1904-75W	0.031	659	15%	1.5
US Biological, clone 8L354, I1904-75W	0.008	526	36%	1.2
US Biological, clone 8L354, I1904-75W	0.002	371	22%	0.9
US Biological, clone 8L354, I1904-75W	0.000	426	13%	1.0

2.5 Effect of AP Conjugate Stabilizers

The effect of detector diluents was tested using Southern Biotech clone JDC-10 at a concentration of 100 ng/mL and 10-10-10 protocol with a post sample wash. Theranos AP Conjugate Stabilizer was the performer.

Table 6: Effect of Detector Diluents

Diluent	Dilution	AVG RLU	CV	Modulation
Assay Buffer (3% BSA)	0.500	444258	15%	457.6
Assay Buffer (3% BSA)	0.125	122889	29%	126.6
Assay Buffer (3% BSA)	0.031	38228	13%	39.4
Assay Buffer (3% BSA)	0.008	10537	24%	10.9
Assay Buffer (3% BSA)	0.002	3122	13%	3.2
Assay Buffer (3% BSA)	0.000	971	23%	1.0
Theranos AP Conjugate Stabilizer	0.500	400070	22%	615.5
Theranos AP Conjugate Stabilizer	0.125	129092	34%	198.6
Theranos AP Conjugate Stabilizer	0.031	46586	14%	71.7
Theranos AP Conjugate Stabilizer	0.008	11199	34%	17.2
Theranos AP Conjugate Stabilizer	0.002	4418	16%	6.8
Theranos AP Conjugate Stabilizer	0.000	650	27%	1.0
Biostab	0.500	1013516	7%	453.5
Biostab	0.125	412943	8%	184.8
Biostab	0.031	148129	19%	66.3
Biostab	0.008	48110	11%	21.5
Biostab	0.002	13870	23%	6.2
Biostab	0.000	2235	28%	1.0
Stabilzyme AP	0.500	264850	23%	304.3
Stabilzyme AP	0.125	59095	32%	67.9
Stabilzyme AP	0.031	13747	18%	15.8
Stabilzyme AP	0.008	5305	18%	6.1
Stabilzyme AP	0.002	1099	64%	1.3
Stabilzyme AP	0.000	870	28%	1.0

2.6 Effect of Reagent Incubation Times

The optimum assay incubation time was determined by testing four post sample wash protocols (10-10-10, 5-5-5, 2-2-1, and 1-1-1) on the Theranos system. The detector used was from Southern Biotech clone JDC-10 at a concentration of 100 ng/mL. The 5-5-5 (sample-detector-substrate) incubation time was best.

Table 7: Assay Protocols

Protocol	Dilution	AVG RLU	CV	Modulation
Generic2_10x_PSW_10-10-10	0.500	1363363	15%	242.2
Generic2_10x_PSW_10-10-10	0.125	593748	13%	105.5
Generic2_10x_PSW_10-10-10	0.031	224772	12%	39.9
Generic2_10x_PSW_10-10-10	0.008	64558	20%	11.5
Generic2_10x_PSW_10-10-10	0.002	20442	10%	3.6
Generic2_10x_PSW_10-10-10	0.000	5629	11%	1.0
Generic2_10X_PSW_5-5-5	0.500	612901	15%	331.4
Generic2_10X_PSW_5-5-5	0.125	208464	18%	112.7
Generic2_10X_PSW_5-5-5	0.031	58276	19%	31.5
Generic2_10X_PSW_5-5-5	0.008	19051	6%	10.3
Generic2_10X_PSW_5-5-5	0.002	5408	32%	2.9
Generic2_10X_PSW_5-5-5	0.000	1849	16%	1.0
Generic2_10X_PSW_2-2-1	0.500	84927	17%	262.7
Generic2_10X_PSW_2-2-1	0.125	26253	23%	81.2
Generic2_10X_PSW_2-2-1	0.031	6963	24%	21.5
Generic2_10X_PSW_2-2-1	0.008	2224	22%	6.9
Generic2_10X_PSW_2-2-1	0.002	673	22%	2.1
Generic2_10X_PSW_2-2-1	0.000	323	18%	1.0
Generic2_10X_PSW_1-1-1	0.500	36040	21%	105.4
Generic2_10X_PSW_1-1-1	0.125	8085	17%	23.7
Generic2_10X_PSW_1-1-1	0.031	2847	21%	8.3
Generic2_10X_PSW_1-1-1	0.008	1069	19%	3.1
Generic2_10X_PSW_1-1-1	0.002	582	23%	1.7
Generic2_10X_PSW_1-1-1	0.000	342	12%	1.0

2.7 Detector Antibody Titration

The optimum detector concentration was determined by titrating Southern Biotech clone JDC-10 at 200 ng/mL, 100 ng/mL, 50 ng/mL, and 25 ng/mL in in-house buffer (Zn^{2+}/Mg^{2+}). The protocol was 10x sample dilution with 5-5-5- incubation times. Best modulations were achieved with 100 ng/mL of detector antibody.

Table 8: Detector Antibody Titration

Detector	Dilution	AVG RLU	CV	Modulation
200 ng/mL	0.500	868805	12%	800.3
200 ng/mL	0.125	204519	11%	188.4
200 ng/mL	0.031	79922	16%	73.6
200 ng/mL	0.008	20437	11%	18.8
200 ng/mL	0.002	8136	17%	7.5
200 ng/mL	0.000	1086	26%	1.0
100 ng/mL	0.500	493860	24%	907.0
100 ng/mL	0.125	157434	9%	289.1
100 ng/mL	0.031	39605	19%	72.7
100 ng/mL	0.008	11669	10%	21.4
100 ng/mL	0.002	3551	15%	6.5
100 ng/mL	0.000	545	16%	1.0
50 ng/mL	0.500	312054	9%	717.3
50 ng/mL	0.125	84375	25%	194.0
50 ng/mL	0.031	19631	21%	45.1
50 ng/mL	0.008	5961	11%	13.7
50 ng/mL	0.002	2031	26%	4.7
50 ng/mL	0.000	435	15%	1.0
25 ng/mL	0.500	158099	17%	629.2
25 ng/mL	0.125	40800	28%	162.4
25 ng/mL	0.031	11060	20%	44.0
25 ng/mL	0.008	3648	14%	14.5
25 ng/mL	0.002	1040	9%	4.1
25 ng/mL	0.000	251	8%	1.0

2.8 Effect of Assay Diluents

The effect of assay diluents was tested by comparing 3% assay buffer to starting block and super block. The protocol used was 10x sample dilution with 5-5-5 incubation times. The Southern Biotech detector antibody was at 100 ng/mL. Although starting block gave the best modulations, assay buffer alone was sufficient.

Table 9: Effect of Assay Diluents

Diluent	Dilution	AVG RLU	CV	Modulation
Assay Buffer	0.500	481027	16%	762.2
Assay Buffer	0.125	150939	22%	239.2
Assay Buffer	0.031	43164	12%	68.4
Assay Buffer	0.008	12171	20%	19.3
Assay Buffer	0.002	3775	14%	6.0
Assay Buffer	0.000	631	20%	1.0
Starting Block	0.500	481252	31%	1259.2
Starting Block	0.125	156428	26%	409.3
Starting Block	0.031	41527	22%	108.7
Starting Block	0.008	13544	16%	35.4
Starting Block	0.002	3811	11%	10.0
Starting Block	0.000	382	9%	1.0
Super Block	0.500	459305	9%	767.8
Super Block	0.125	164005	10%	274.2
Super Block	0.031	35689	26%	59.7
Super Block	0.008	10473	28%	17.5
Super Block	0.002	2863	18%	4.8
Super Block	0.000	598	14%	1.0

2.9 New Assay Protocols

The assay protocol was retested after receiving the anti-TPO WHO (66/387, NIBSC) standard. The sample dilution was increased to 100x to avoid saturation, and different incubation times were tested again with the post sample wash. The 100x sample dilution protocol with 2-2-1 incubation times was most linear and gave acceptable sensitivity. The WHO standard was diluted in assay buffer.

Table 10: 100x PSW Protocols

Protocol	Anti-TPO [IU/mL]	AVG RLU	CV	Modulation
Generic2_100x_PSW_10-10-10	500.0	1597837	10%	3371
Generic2_100x_PSW_10-10-10	166.7	1077299	10%	2273
Generic2_100x_PSW_10-10-10	55.6	639418	10%	1349
Generic2_100x_PSW_10-10-10	18.5	245729	19%	518
Generic2_100x_PSW_10-10-10	6.2	92762	23%	196
Generic2_100x_PSW_10-10-10	2.1	32627	26%	69
Generic2_100x_PSW_10-10-10	0.7	14342	18%	30
Generic2_100x_PSW_10-10-10	0.0	474	20%	1

Protocol	Anti-TPO [IU/mL]	AVG RLU	CV	Modulation
Generic2_100x_PSW_5-5-5	500	894111	8%	3580
Generic2_100x_PSW_5-5-5	166.7	477637	26%	1912
Generic2_100x_PSW_5-5-5	55.6	281607	5%	1127
Generic2_100x_PSW_5-5-5	18.5	73374	13%	294
Generic2_100x_PSW_5-5-5	6.2	33134	24%	133
Generic2_100x_PSW_5-5-5	2.1	10900	18%	44
Generic2_100x_PSW_5-5-5	0.7	3795	11%	15
Generic2_100x_PSW_5-5-5	0.0	250	12%	1

Protocol	Anti-TPO [IU/mL]	AVG RLU	CV	Modulation
Generic2_100x_PSW_2-2-1	500.0	159765	21%	1639
Generic2_100x_PSW_2-2-1	166.7	61647	25%	632
Generic2_100x_PSW_2-2-1	55.6	21795	18%	224
Generic2_100x_PSW_2-2-1	18.5	5091	22%	52
Generic2_100x_PSW_2-2-1	6.2	2703	22%	28
Generic2_100x_PSW_2-2-1	2.1	1105	27%	11
Generic2_100x_PSW_2-2-1	0.7	390	21%	4
Generic2_100x_PSW_2-2-1	0.0	97	6%	1

2.10 Effect of Sample Dilutions

The assay was also tested with the 1000x sample dilution protocol with 5-5-5 incubation times. The sensitivity and precision at 1000x sample dilution was comparable to previous results with the 100x sample dilution and 2-2-1 protocol.

Table 11: 1000x PSW Protocol

Protocol	Anti-TPO [IU/mL]	AVG RLU	CV	Modulation
Generic2_1000x_PSW_5-5-5	500.0	134292	23%	630
Generic2_1000x_PSW_5-5-5	166.7	61949	20%	291
Generic2_1000x_PSW_5-5-5	55.6	21735	24%	102
Generic2_1000x_PSW_5-5-5	18.5	6828	30%	32
Generic2_1000x_PSW_5-5-5	6.2	3130	15%	15
Generic2_1000x_PSW_5-5-5	2.1	1390	15%	7
Generic2_1000x_PSW_5-5-5	0.7	645	16%	3
Generic2_1000x_PSW_5-5-5	0.0	213	11%	1

2.11 Matrix Effects

The matrix was tested by spiking the anti-TPO WHO standard into whole blood, EDTA plasma, and Li-Heparin plasma. The protocols tested were 100x sample dilution with post sample wash and 2-2-1 incubation and 1000x sample dilution with post sample wash and 5-5-5 incubation. The detector was used at 100 ng/mL. Recoveries with the 1000x sample dilution protocol were better than the 100x sample dilution protocol. There was minimal anti-coagulant effects. The final assay protocol was 1000x sample dilution with post sample wash and 5-5-5 incubation times.

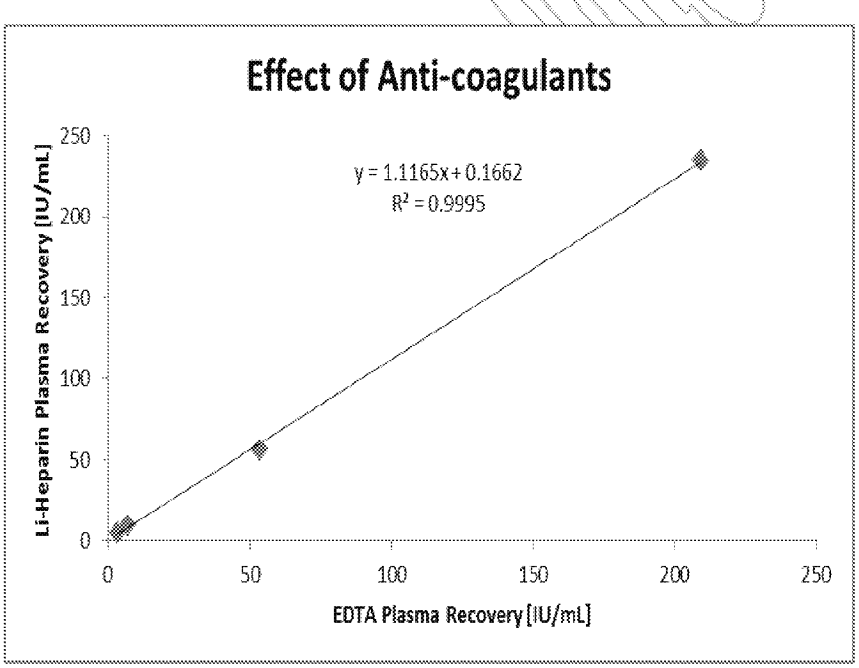
Table 12: 100x sample dilution with PSW and 2-2-1 incubation

Description	Spiked [IU/mL]	AVG RLU	CV	Back-Cal [IU/mL]	Recovery
Whole Blood EDTA	250	30241	55%	34.9	14%
Whole Blood EDTA	50	13187	21%	13.2	26%
Whole Blood EDTA	5	1324	21%	-0.5	-10%
Whole Blood EDTA	0	290	19%	-1.7	
EDTA Plasma	250	40140	29%	48.6	19%
EDTA Plasma	50	6511	35%	5.4	11%
EDTA Plasma	5	703	22%	-1.2	-24%
EDTA Plasma	0	297	16%	-1.7	
Li-Heparin Plasma	250	54117	26%	69.3	28%
Li-Heparin Plasma	50	7942	15%	7	14%
Li-Heparin Plasma	5	986	13%	-0.9	-18%
Li-Heparin Plasma	0	302	21%	-1.6	

Table 13: 1000x sample dilution with PSW and 5-5-5 incubation

Description	Spiked [IU/mL]	AVG RLU	CV	Back-Cal [IU/mL]	Recovery
Whole Blood EDTA	250	113583	7%	247.2	98%
Whole Blood EDTA	50	26955	19%	56.6	110%
Whole Blood EDTA	5	4469	24%	7.1	107%
Whole Blood EDTA	0	2030	10%	1.8	n/a
EDTA Plasma	250	96309	20%	209.2	82%
EDTA Plasma	50	25491	17%	53.4	100%
EDTA Plasma	5	4395	25%	7.0	75%
EDTA Plasma	0	2700	7%	3.3	n/a
Heparin Plasma	250	107831	19%	234.5	92%
Heparin Plasma	50	26807	12%	56.3	103%
Heparin Plasma	5	5709	14%	9.9	106%
Heparin Plasma	0	3296	7%	4.6	n/a

Figure 1: Effect of Anti-coagulants



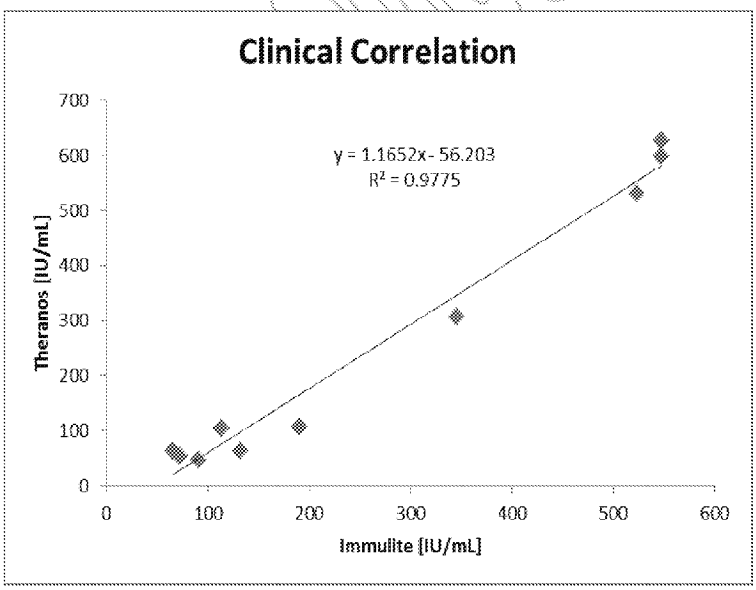
2.12 Clincial Correlation

The assay clinical correlation was tested by assaying clinical samples from Bioreclamation under finalized assay conditions. The Theranos results were comparable to the Bioreclamation reported results (Siemens, Immulite). The overall correlation was good with an R^2 value of 0.98.

Table 14: Clinical Correlation

Sample	Theranos [IU/mL]	Bioreclamation [IU/mL]
1	54.5	72.6
2	46.4	90.7
3	598.4	548
4	530.9	523
5	3732.9	>1000
6	106.7	190
7	104.2	113
8	63.0	132
9	7541.2	>1000
10	62.6	66
11	626.5	548
12	307.3	345

Figure 2: Clinical Correlation



2.13 Cutoff Value

The cutoff value of the assay was determined by testing 36 confirmed negative anti-TPO samples on the Theranos system. The cutoff value was 8.26 IU/mL (mean + 2SD). The published reference interval for anti-TPO was 0 – 9 IU/mL (Arup Laboratories). The lowest confirmed negative value was 12.59 IU/mL. The cutoff ranges from 20 to 40 IU/mL for commercial assays. The suggested cutoff value was 35 IU/mL.

Table 15: Cutoff Value

NO.	anti-TPO [IU/mL]
1	4.96
2	3.83
3	2.61
4	6.01
5	3.25
6	6.44
7	4.42
8	6.47
9	3.97
10	3.03
11	0.99
12	2.55
13	6.87
14	12.59
15	0.51
16	3.29
17	2.07
18	1.95
19	0.83
20	1.37
21	5.71
22	4.39
23	4.41
24	5.1
25	1.87
26	2.72
27	2.07
28	1.04
29	2.25
30	2.28
31	1.69
32	2.20
33	1.90
34	2.43
35	2.25
36	7.71
37	0.95
38	2.71
AVG	3.47
STD	2.40
2*STD	4.80
Expected Value	8.26

2.14 HAMA and RF Samples

The assay was tested for HAMA and RF interference. There was no obvious interference from the HAMA and RF samples. The results were confirmed with the DRG ELISA kit.

Table 16: HAMA and RF Samples

		Theranos System		DRG Kit	
		cutoff = 35 IU/mL		cutoff = 40 IU/mL	
Description	Sample	anti-TPO [IU/mL]	Result	anti-TPO [IU/mL]	Result
HAMA	H1	4.96	Negative	9.22	Negative
HAMA	H2	3.83	Negative	7.73	Negative
HAMA	H3	2.61	Negative	8.18	Negative
HAMA	H4	6.01	Negative	13.85	Negative
HAMA	H5	3.25	Negative	10.04	Negative
HAMA	H6	6.44	Negative	14.20	Negative
RF	RF1	0.99	Negative	17.16	Negative
RF	RF2	2.55	Negative	8.53	Negative
RF	RF3	6.87	Negative	8.86	Negative
RF	RF4	12.59	Negative	11.00	Negative
RF	RF5	0.51	Negative	6.35	Negative
RF	RF6	3.29	Negative	12.43	Negative

2.15 ANA Samples

The assay was tested for ANA interference. Two out of the 10 samples tested were positive. These samples were also positive on the Siemens Immulite. Overall, there was no obvious interference from ANA samples.

Table 17: ANA Samples

		Theranos System		Immulite	
		cutoff = 35 IU/mL		cutoff = 35 IU/mL	
Description	Sample	anti-TPO [IU/mL]	Result	anti-TPO [IU/mL]	Result
ANA	1	5.7	Negative	< 10.0	Negative
ANA	2	4.4	Negative	10.5	Negative
ANA	3	4.4	Negative	31.6	Negative
ANA	4	5.1	Negative	18.4	Negative
ANA	5	290.9	Positive	575	Positive
ANA	6	1.9	Negative	11.2	Negative
ANA	7	141.8	Positive	278	Positive
ANA	8	530.7	Positive	391	Positive
ANA	9	2.7	Negative	< 10.0	Negative
ANA	10	2.1	Negative	11.7	Negative

2.16 Thyroglobulin Interference

The assay was also tested for thyroglobulin interference. Thyroglobulin at 25 ug/mL was spiked into the standard curve and the back-calculated results compared to the expected values. The recoveries were good in the presence of thyroglobulin.

Table 18: Thyroglobulin Interference

	+ TG	
Expected	Observed	Recoveries
500.00	468.69	94%
166.67	161.77	97%
55.56	57.79	104%
18.52	23.36	126%
6.17	5.93	96%
2.06	2.41	117%
0.69	0.67	98%
0.00	0.00	

3. ASSAY SUMMARY

Table 19: Development Summary

Capture Antigen	Biotin TPO @ 1 ug/mL
Wash Buffer	1X Enzo from 20X
Assay Buffer	3% BSA in TBS
Edison Protocol	Generic2_1000X_PSW_5-5-5
Detector Antibody	Southern Biotech clone JDC-10 @ 25 ng/mL
Detector Stabilizer	Theranos AP Conjugate Stabilizer
Sample Dilution	1000X

4. CLINICAL EVALUATION

To further validate the assay, more normal and positive samples need to be tested. The cutoff value needs to be verified by screening more normal patients. At least 100 or more patients are needed for clinical evaluation.