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Total Triiodothyronine (TT3) 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 total Triiodothyronine (T3) in human whole blood (automatically processed into plasma by the Theranos Analyzer), plasma and serum. The assay has a reportable normal range of 0.2 ng/ml to 10.2 ng/ml, and is calibrated to the European Commission Certified Reference Material IRMM-469.

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

The following commercial ELISA kits have been used in house for comparison:
1. Calbiotech (Cat #T3104T) and
2. Immuno-Biological Laboratories, Inc.(Cat# IB69112).

1.1.2 Materials and methods

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

In this assay format, the T3 in the sample competed with T3-AP for binding to the anti-T3 antibody. An anti-mouse antibody served as the capture surface for the competitive ELISA. A dissociating agent acid was used to help release T3 from its binding proteins. Labeled Triiodothyronine (T3-AP) served as the tracer. The mixture was incubated with the capture surface followed by wash steps. Then the substrate is incubated with the capture surface. The resulting signal was read in Relative Light Units (RLU).

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

2.1 Cross Reactivity and Interference

To test cross reactivity and interference for the antibody, 7 different substances that could potentially cross react or interfere with T3 were tested. The test levels chosen for each test substance were three times higher than the highest level present/administered to a patient in presence of a range of T3. The recovery of T3 was calculated on the control standard curve.

No cross reactivity was observed with any of the substances tested. Over recovery with phenylbutazone is acceptable as the CDC states that anti-inflammatory substances such as this are known to interfere with equilibrium levels of total T3 and free T3 as they play similar roles to displace T3 from its binding proteins.

Table [SEQ Table * ARABIC]: Cross Reactivity and Interference

Test Substance	[Test Substance] ng/ml	[T3] ng/ml	Signal RLU		Back-Calculated Conc. (ng/mL)		
			Mean RLU	CV%	Mean Conc.	CV%	%Recovery
Control		10.2	892	4.3	10.4	4.1	102
		5.2	2465	15.2	4.9	9.1	95
		1.7	9997	4.4	1.9	3.8	113
		1.2	16967	3.1	1.1	3.7	93
		0.6	24548	3	0.7	4.3	112
Thyroxine (T4)	900	10.2	1093	2.7	8.6	2.3	85
		5.2	2209	7.9	5.2	5.1	101
		1.2	13854	1.3	1.4	1.4	117
3,3',5' Triiodothyronine (Reverse T3)	1.5	10.2	1165	0.5	8.2	0.4	80
		5.2	2665	13.8	4.7	7.9	90
		1.2	16081	5.8	1.2	6.9	99
3-Iodo-L-tyrosine	30	10.2	962	5.9	9.7	5.3	95
		5.2	2430	3.2	4.9	2.0	94
		1.2	13117	4.7	1.5	4.8	124
3,5-Diiodo-L-thyronine (T2)	30	10.2	1111	5.9	8.5	5.0	84
		5.2	3128	0.4	4.6	0.2	89
		1.2	13489	4.1	1.4	4.2	120

Cross Reactivity and Interference (continued)

Test Substance	[Test] ng/ml	[T3] ng/ml	Signal (RLU) Mean RLU	Signal (RLU) CV%	Back Calculated Conc. (ng/mL) Mean Conc.	Back Calculated Conc. (ng/mL) Conc CV%	Back Calculated Conc. (ng/mL) %Recovery
Sodium Salicylate	400000	10.2	1152	10.2	8.3	8.7	82
		5.2	2415	4.5	4.9	2.7	95
		1.2	14781	2.7	1.3	3.0	109
PhenylButazone	300000	10.2	928	15.8	10.2	14.8	100
		5.2	1998	1.6	5.3	1.1	102
		1.2	10719	8.8	1.8	8.2	151
Diphenylhydantoin	300	10.2	1170	5.4	8.2	4.4	80
		5.2	2510	7.5	4.8	2.9	92
		1.2	14935	8.9	1.3	9.6	108

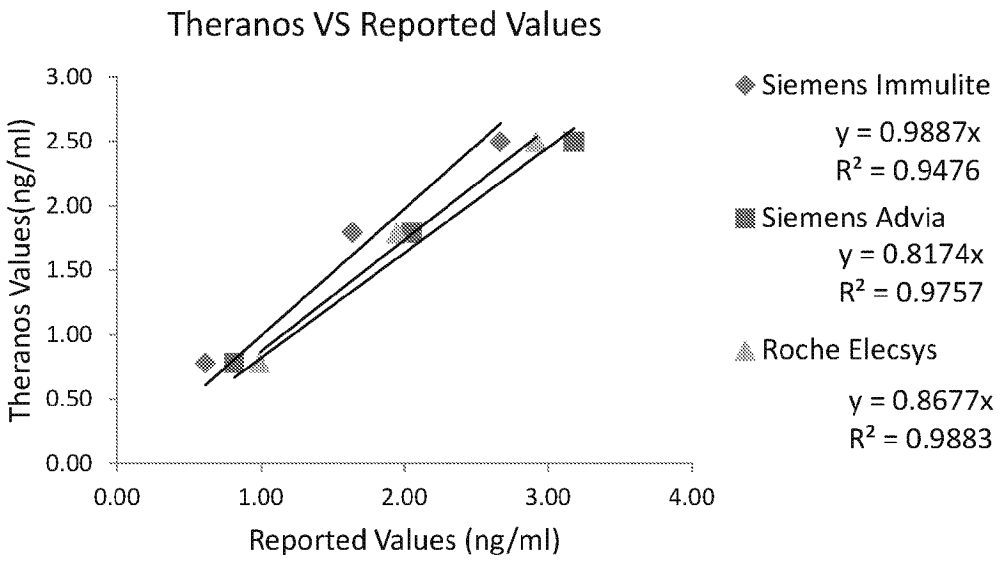
2.2 Calibration Verification

Two calibration verification methods were tested on the Theranos Analyzer. One of these methods involved obtaining three level serum controls from BioRad (Liquichek Immunoassay Plus) and measuring it on the Theranos Analyzer. Results were compared to those reported by the clinical analyzers. The controls correlate very well with the Siemens Immulite 2000/2500.

Table [SEQ Table * ARABIC]: BioRad Liquichek Immunoassay Plus Controls Reported Mean Values

Biorad Control	Mean Reported Result (ng/mL)			
	Theranos 3.0 System	Siemens Immulite 2000/2500	Siemens Advia Centaur XP	Roche Elecsys/E170/COBAS E SYSTEMS
Level1	0.78	0.61	0.81	0.994
Level 2	1.79	1.64	2.05	1.95
Level 3	2.50	2.67	3.18	2.92

Figure [SEQ Figure * ARABIC]: Calibration Verification-BioRad Liquichek Immunoassay Plus Controls



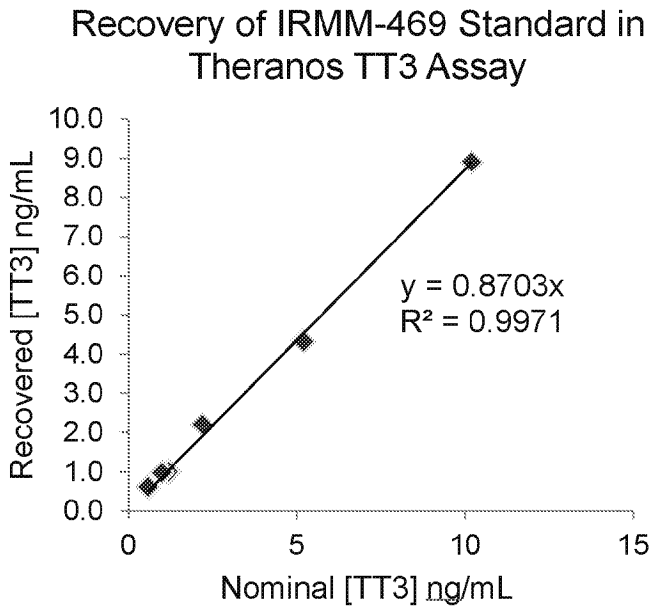
The second method of calibration verification for the Theranos Analyzer was performed by testing the European Commission Certified Reference Material for T3 (IRMM-469). This involved reconstituting the reference material according to the supplied directions, and making

dilutions into the same depleted serum as the Theranos calibrators. Recovery of the reference material and the controls was excellent (Table 19, Figure 2).

Table [SEQ Table * ARABIC]: Recovery of IRMM-469(T3) in depleted serum

	Signal (RLU)		Back Calculated Conc. (ng/mL)		
[T3] ng/ml	Mean RLU	CV%	Mean Conc.	CV%	%Recovery
10.2	864	5.0	8.9	5.8	87
5.2	1937	4.0	4.3	2.6	83
2.2	5808	2.8	2.2	2.0	101
1.2	13002	3.3	1.0	4.4	83
1	13197	8.2	1.0	11.2	98
0.6	18193	16.5	0.6	31.1	103

Figure [SEQ Figure * ARABIC]: Calibration Verification-IRMM-469



2.3 Clinical Correlation

Serum samples from pateints with either known T4 or TSH or T3 Uptake reported values were run on the Calbiotech kit to obtain the total T3 values. While a range of low and normal T3 values were obtained this way, there were no clinical samples with high values of total T3. To obtain samples with high T3 values,normal serum samples were spiked with high levels of T3 analyte. The total T3 values for these spiked samples were obtained similarly by running them in the Calbiotech kit. A set of 23 clinicals samples across the T3 range were selected to run on the Theranos Analyzer for the clinical correlation analysis. The T3 values obtained from Calbiotech kit correlated very well to the values obtained from the Theranos Analyzer.

Table [SEQ Table * ARABIC]: Standard Curve

[T3] ng/ml	Signal (RLU)		Back-Calculated Conc. (ng/ml)		
	Mean RLU	CV%	Mean Conc.	CV%	% Recovery
10.2	764	14.7	10.8	17.1	105
5.2	1573	12.7	5.1	10.7	98
2.2	5198	9.7	2.4	7.1	109
1.7	7882	12.8	1.7	11.5	102
1.2	11853	9.5	1.1	11.7	94
1	13404	10.6	1.0	14.3	96
0.6	17121	4.1	0.7	6.8	110
0.2	30162	6.0	0.2	14.5	102

Figure [SEQ Figure * ARABIC]: Standard Curve-Theranos Analyzer

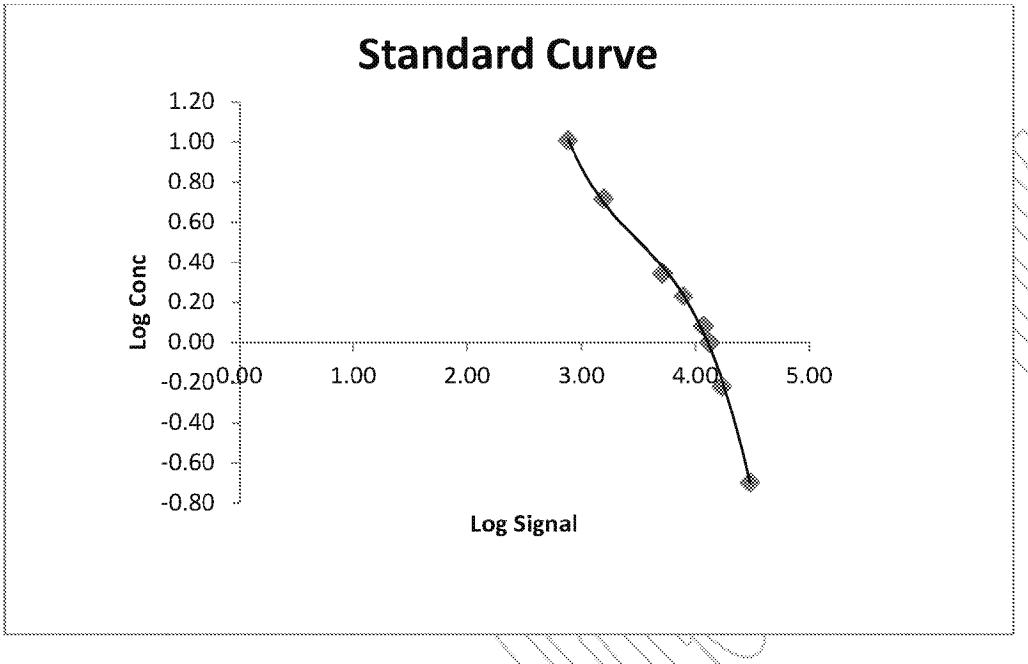


Figure [SEQ Figure * ARABIC]: Clinical correlation-Theranos Analyzer

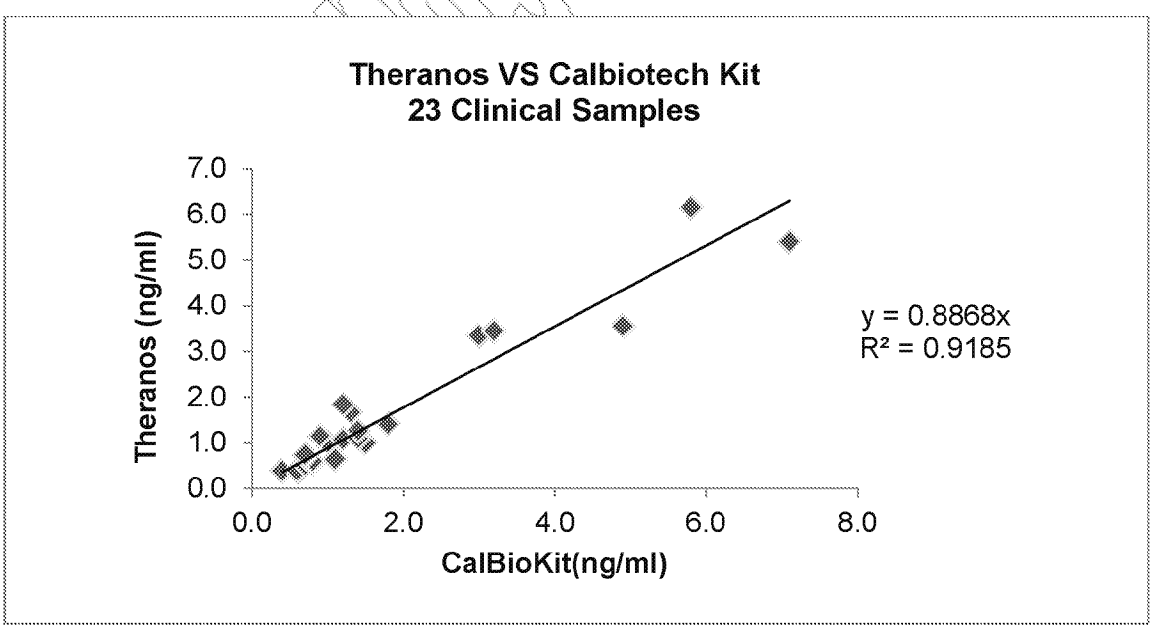


Table [SEQ Table * ARABIC]: Clinical Sample Data from the Calbiokit and the Theranos Analyzer

SAMPLE	Calbiotech Kit, ng/mL	Theranos, ng/mL
1	0.6	0.4
2	0.4	0.4
3	0.6	0.4
4	0.7	0.5
5	0.8	0.6
6	0.8	0.6
7	1.1	0.6
8	0.7	0.7
9	0.7	0.7
10	1.5	1.0
11	1.0	1.0
12	1.2	1.1
13	1.4	1.1
14	0.9	1.1
15	1.4	1.3
16	1.8	1.4
17	1.3	1.7
18	1.2	1.8
19	3.0	3.3
20	3.2	3.4
21	4.9	3.6
22	7.1	5.4
23	5.8	6.2

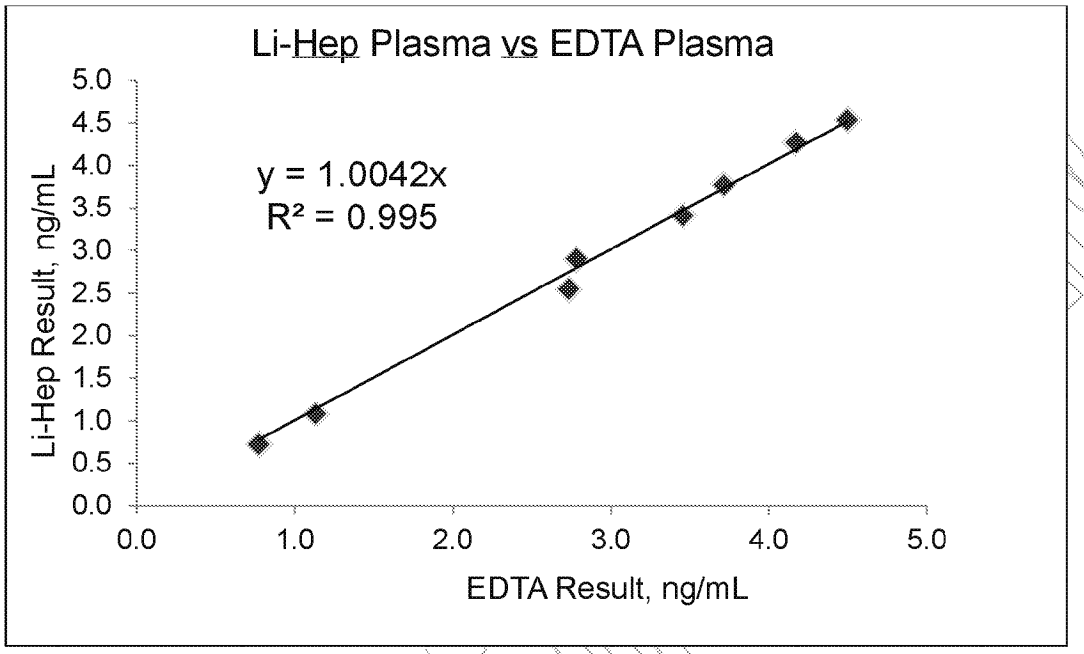
2.4 Effects of Anticoagulant

The Theranos Analyzer can prepare plasma from both EDTA and lithium-heparin-treated blood. Matched plasma samples were prepared from both EDTA tubes and from Li-Hep tubes for 3 normal donors.

Table [SEQ Table * ARABIC]: Effect of Anticoagulant

Sample	Type	Spiked [T3] ng/ml	Signal (RLU)		Concentration (ng/mL)			
			Mean RLU	CV%	Mean Conc.	CV%	Minus Endogenous	% Recovery
1	Li-Hep	4.2	1873	2.4	4.3	1.6	3.2	75
		3.2	2655	2.6	3.4	1.7	2.3	73
		2.1	3510	23.0	2.9	16.3	1.8	85
		0	10171	4.8	1.1	6.2	0.0	
	EDTA	4.2	1959	12.1	4.2	8.0	3.0	72
		3.2	2600	2.1	3.5	1.4	2.3	73
		2.1	3645	9.9	2.8	6.6	1.7	77
		0	9810	1.9	1.1	2.4	0.0	
2	Li-Hep	4.2	1666	4.2	4.5	2.6	3.8	90
		3.2	2274	6.5	3.8	4.2	3.0	96
		2.1	4149	10.5	2.5	7.6	1.8	85
		0	13527	6.8	0.7	10.9	0.0	
	EDTA	4.2	1753	11.3	4.5	7.8	3.7	88
		3.2	2320	3.0	3.7	1.9	2.9	92
		2.1	3748	10.6	2.7	7.2	2.0	92
			12948	4.5	0.8	6.8	0.0	
3	Li-Hep	4.2	1936	2.6	4.2	1.8	3.5	82
		3.2	2673	1.1	3.4	0.7	2.7	84
		2.1	4788	2.1	2.3	1.6	1.6	73
		0	13603	10.0	0.7	15.6	0.0	
	EDTA	4.2	1791	7.7	4.4	5.4	3.8	90
		3.2	2626	11.0	3.4	6.9	2.8	89
		2.1	4529	17.1	2.4	13.8	1.8	83
		0	14781	4.0	0.6	6.7	0.0	

Figure [SEQ Figure * ARABIC]: Effects of anticoagulant



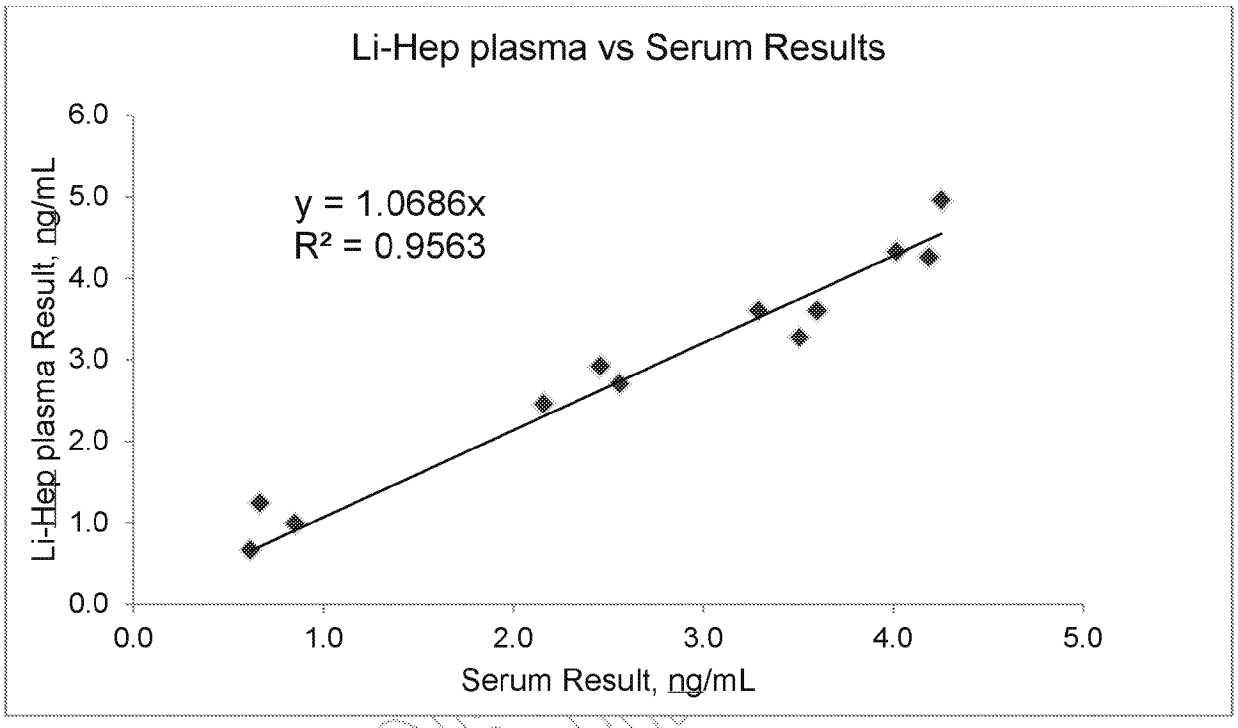
2.5 Matrix Effects: Serum vs Plasma

Theranos Analyzer can test either serum prepared in the lab or plasma prepared on board from a whole blood sample. Matched Serum and Lithium Heparin Plasma from three normal donors were analyzed.

Table [SEQ Table * ARABIC]: Lithium-Heparin Plasma vs. Serum

Sample	Type	Spike [T3] ng/ml	Signal (RLU) Mean RLU	CV%	Concentration (ng/mL) Mean Conc.	Conc CV%	Minus Endogenous	% Recovery
1	Serum	4.2	2056	3.7	4.0	2.4	3.2	76
		3.3	2581	16.2	3.5	10.4	2.7	81
		2.1	4100	5.7	2.6	3.9	1.7	82
		0	11873	4.8	0.8	3.8	0.0	
	Plasma	4.2	1841	6.3	4.3	4.2	3.3	80
		3.3	2853	9.9	3.3	6.4	2.3	70
		2.1	3782	1.7	2.7	1.2	1.7	82
		0	10901	5.7	1.0	7.5	0.0	
2	Serum	4.2	1929	3.0	4.2	2.0	3.6	85
		3.3	2823	10.8	3.3	6.8	2.7	82
		2.1	5183	14.1	2.2	11.4	1.5	73
		0	14971	9.0	0.6	15.8	0.0	
	Plasma	4.2	1887	4.7	4.3	3.1	3.6	85
		3.3	2440	4.7	3.6	3.1	2.9	90
		2.1	4342	6.2	2.5	4.5	1.8	85
		0	14208	3.5	0.7	5.8	0.0	
3	Serum	4.2	1887	4.7	4.3	3.1	3.6	85
		3.3	2440	4.7	3.6	3.1	2.9	90
		2.1	4342	6.2	2.5	4.5	1.8	85
		0	14208	3.5	0.7	5.8	0.0	
	Plasma	4.2	1526	9.8	5.0	6.8	3.7	88
		3.3	2448	6.4	3.6	4.0	2.4	72
		2.1	3402	11.3	2.9	7.6	1.7	80
		0	9093	1.0	1.2	1.2	0.0	

Figure [SEQ Figure * ARABIC]: Matrix Effects: Lithium-Heparin Plasma vs Serum



2.6 Interfering Matrix Effects

Spike recovery was tested in lipemic, icteric and hemolyzed serum obtained from ProMedDx to ascertain whether there may be interference in the assay results when measuring these types of samples. ARUP states that hemolyzed samples are completely unacceptable; however, the recovery for these samples on the Theranos Analyzer is still fair, though results may be slightly under-reported in grossly hemolyzed samples. Lipemic and icteric samples do not seem to interfere for this assay.

Table [SEQ Table * ARABIC]: Spike Recovery in Lipemic Sera

Sample Type	Spiked [T3] ng/ml	Signal (RLU)		Concentration (ng/mL)			
		Mean RLU	CV%	Mean Conc.	CV%	Minus Endogenous	% Recovery
Lipemic-S1	4.2	1323	9.2	5.5	7.6	3.7	88
	3.3	1695	4.4	4.6	3.1	2.8	85
	2.1	2583	9.6	3.5	6.4	1.7	80
	0	6358	9.5	1.8	8.8	0.0	
Lipemic-S2	4.2	1701	10.2	4.6	7.1	3.3	79
	3.3	1922	8.8	4.2	5.9	2.9	90
	2.1	3418	4.8	2.9	3.2	1.6	77
	0	8857	3.7	1.3	4.3	0.0	

Figure [SEQ Figure * ARABIC]: Spike Recovery in Lipemic Sera

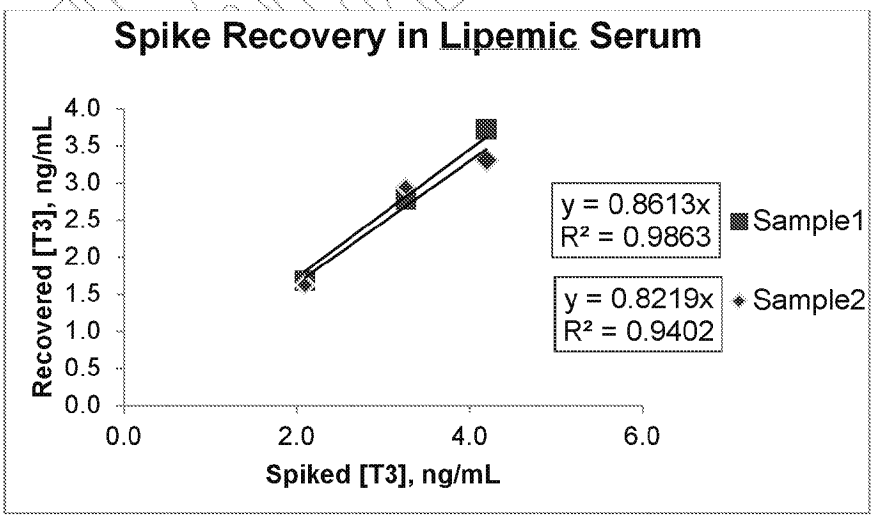


Table [SEQ Table * ARABIC]: Spike Recovery in Icteric Sera

Sample Type	Spiked [T3] ng/ml	Signal (RLU)		Concentration (ng/mL)			
		Mean RLU	CV%	Mean Conc.	CV%	Minus Endogenous	% Recovery
Icteric-S1	4.2	1834	10.3	4.4	6.9	3.6	86
	3.3	2279	6.6	3.8	4.2	3.0	93
	2.1	3823	5.7	2.7	3.8	1.9	93
	0	13253	2.6	0.7	4.1	0.0	
Icteric-S2	4.2	1884	5.6	4.3	3.7	3.2	77
	3.3	2194	3.1	3.9	2.0	2.8	86
	2.1	4610	2.3	2.3	1.7	1.3	62
	0	10494	3.0	1.0	3.8	0.0	
Icteric-S3	4.2	1664	12.8	4.7	8.8	3.7	88
	3.3	2323	1.1	3.7	0.7	2.7	84
	2.1	4171	4.1	2.5	3.0	1.6	74
	0	11106	8.2	1.0	11.2	0.0	

Figure [SEQ Figure * ARABIC]: Spike Recovery in Icteric Sera

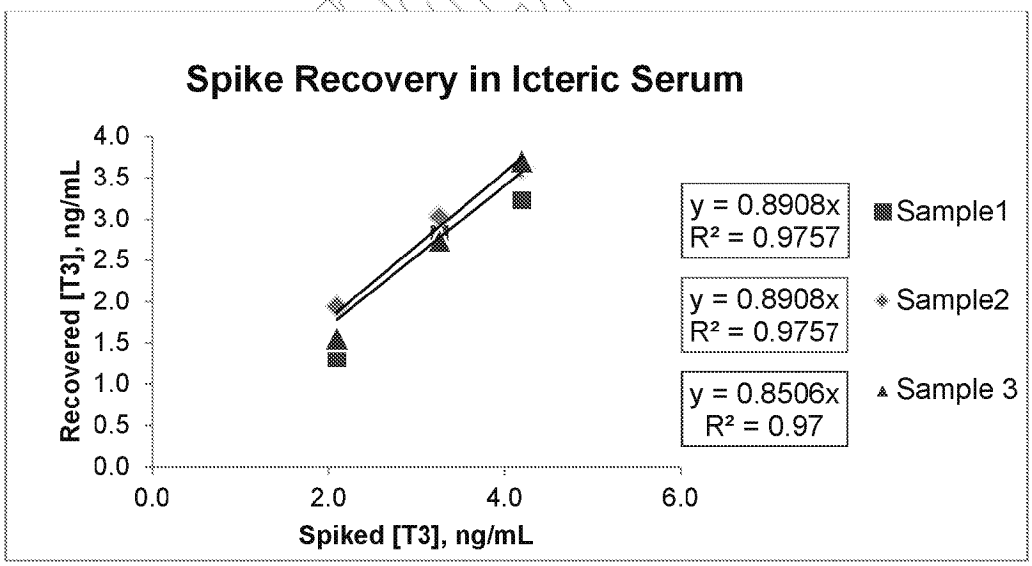
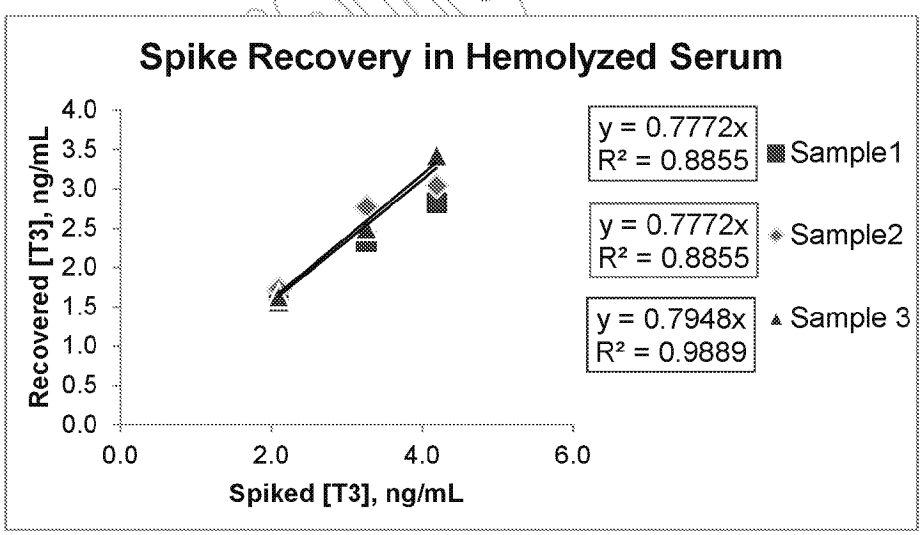


Table [SEQ Table * ARABIC]: Spike recovery in Hemolyzed samples

Sample Type	Spiked [T3] ng/ml	Signal (RLU)		Concentration (ng/ml)			
		Mean RLU	CV%	Mean Conc.	CV%	Minus Endogenous	% Recovery
Hemolyzed -S1	4.2	2666	12.4	3.4	7.9	2.8	67
	3.3	3367	2.6	2.9	1.7	2.3	71
	2.1	5095	4.9	2.2	3.9	1.6	75
	0	15111	5.8	0.6	9.9	0.0	
Hemolyzed -S2	4.2	2275	4.3	3.8	2.8	3.0	72
	3.3	2570	10.0	3.5	6.3	2.8	85
	2.1	4365	5.4	2.4	4.0	1.7	82
	0	13605	13.5	0.7	20.8	0.0	
Hemolyzed -S3	4.2	2015	5.0	4.1	3.3	3.4	82
	3.3	3008	3.8	3.1	2.5	2.5	77
	2.1	4769	4.9	2.3	3.7	1.6	78
	0	14440	6.8	0.7	11.1	0.0	

Figure [SEQ Figure * ARABIC]: Spike recovery in Hemolyzed samples



2.7 Stability

Stability of reagents is being monitored.

3 CONCLUSION

We have successfully developed an immunoassay to detect Total T3 in human serum and plasma.