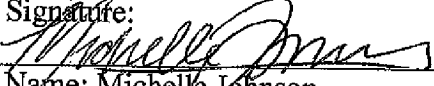
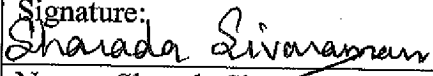


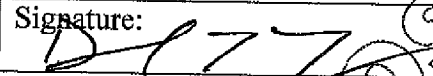
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1 ASSAY BACKGROUND

Triiodothyronine accounts for about 5% of circulating thyroid hormones in serum. It is usually bound to thyroxine-binding globulin (TBG). Thyroid synthesis of T3 is stimulated by TRH release from the hypothalamus, and TSH release from the pituitary. Both T4 and T3 inhibit TSH release from the pituitary and TRH release by the hypothalamus. The Total Triiodothyronine (tT3) test measures the circulating levels of bound and unbound T3. Thyroxine (T4) is deiodinated in the thyroid, as well as the liver to form the more physiologically active T3, and the inactive rT3. It is measured as part of a thyroid function panel which includes TSH and T4 measurements. High levels of fT3 are associated with Grave's disease, hyperthyroidism, painless thyroiditis, thyrotoxic periodic paralysis, and toxic nodular goiter. High fT3 levels not related to thyroid function are also seen in pregnancy and liver disease. Low levels of T3 are associated with hypothyroidism, long term illness, and starvation. Apparent healthy donors should have tT3 levels within the reference range of 84 – 172 ng/dL.

1.1 Theranos System Specifications


This assay is designed to detect total Triiodothyronine (T3) in human EDTA plasma. EDTA plasma is the preferred anticoagulant for this assay. The assay has a reportable range of 47.6 – 1420 ng/dL, and is calibrated to the European Commission Certified Reference Material IRMM-469.

1.2 Reference Assay

The SIEMENS Immulite 2000 Total T3 test was used as a predicate method. The reportable range is 40 – 600 ng/dL. Hemolysis (up to 30uL/mL hemoglobin), lipemia (up to 5000 mg/dL triglycerides), and icterus (up to 200 mg/L bilirubin), does not affect assay precision. EDTA plasma samples are not recommended for testing.

2 REGULATION AND GUIDANCE

The qualification/validation of the ELISA assays on the Theranos device will be in accordance with C.F.R. Ch IV, § 493.1253 "Standard: Establishment and verification of performance specifications" and outlined in CLSI guideline C28A3.

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3 CALIBRATION

3.1 Guidelines

- 3.1.1 In 42 CFR Part 493.1255, it is required to perform calibration procedures with at least the frequency recommended by the manufacturer, or using criteria specified by the laboratory, or when calibration verification fails to meet acceptable limits.
- 3.1.2 For the purposes of this Validation Plan, calibration will be carried out for each new lot of reagent cartridges.
- 3.1.2.1 At each level 3 cartridge replicates were tested. Any individual tip with a value less than 150RLU was considered a "Dark" tip. Any dark tips and outliers were excluded from the mean, %CV and % Recovery calculations.
- 3.1.2.2 Acceptance criteria: For each run, a minimum of 75% points of calibration standards should be within $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ and ULOQ standards) of their nominal value, and a minimum of six unique standard concentrations must be within the assay range.

3.2 Calibration 1 (Edison 3.5, 200L)

Of 9 tT3 levels, 8 had a back calculated mean value within $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ and ULOQ standards) of their nominal values, and all levels had CVs below 20% (25% at LLOQ). This calibration set was used to analyze Accuracy/Comparability (5), Dilution Linearity (6), Reference Range (7), Blood Collection Device Comparison (8), Analytical Sensitivity (9), Anticoagulant Comparison (10), Interference (11), and Analyte Stability (13).


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Table 1: Calibration 1 Data

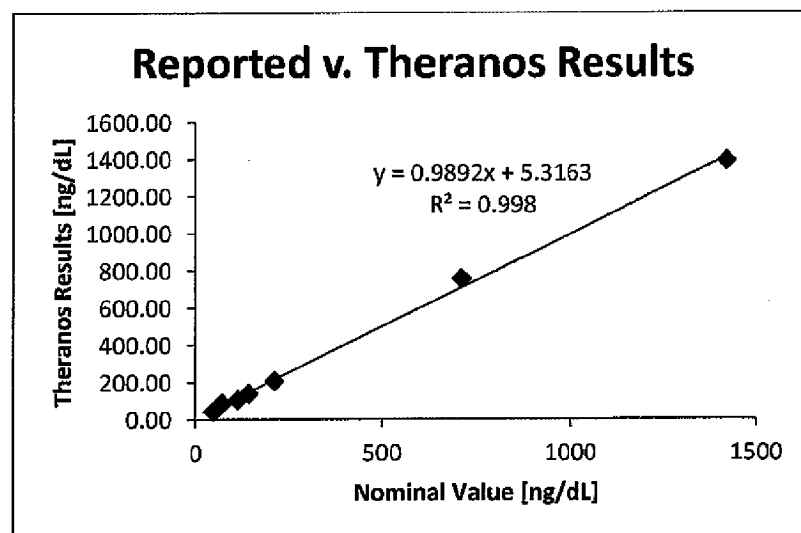
Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV
1420	536	847					1051	16%	1163.95	1319.72	1389.02	16%
	1263	1107	1263	1107	1185	9%			1611.90	1587.53		
	911	924	911	924	918	1%						
710	1920	2001	1920	2001	1961	3%	2084	9%	807.59	780.46	754.71	7%
	2355	2534	2355						683.48			
	1961	2185	1961	2185	2073	8%			793.58	726.23		
213	8215	9194	8215	9194	8705	8%	9709	13%	243.37	218.66	207.23	13%
	41	20	DARK	DARK								
	11042	10385	11042	10385	10714	4%			181.53	193.57		
142	24260	12709		12709			13844	8%		155.30	140.21	9%
	15129	14314	15129	14314	14722	4%			125.18	134.49		
	12788	14281	12788	14281	13535	8%			154.19	134.88		
113.6	15405	15397	15405	15397	15401	0%	17049	8%	122.20	122.28	105.90	12%
	18263	17374	18263	17374	17819	4%			95.25	102.94		
	18246	17608	18246	17608	17927	3%			95.39	100.86		
71	18408	19691	18408	19691	19050	5%	19455	4%	94.05	83.95	85.74	7%
	20318	19402	20318	19402	19860	3%			79.35	86.14		
	30311	29455										
47.6	24262	25961	24262	25961	25112	5%	26585	14%	54.37	45.27	42.13	25%
	21930	25770	21930	25770	23850	11%			68.39	46.26		
	31808	29778	31808	29778	30793	5%			OORL	OORL		
28.4	25079	32522	25079	32522	28801	18%	30303	16%	49.89	OORL	OORL	OORL
	25335	27580	25335	27580	26458	6%			48.53	OORL		
	35092	36212	35092	36212	35652	2%			OORL	OORL		
0	41182	50410	41182	50410	45796	14%	40718	13%	OORL	OORL	OORL	OORL
	40545	36795	40545	36795	38670	7%			OORL	OORL		
	35875	39499	35875	39499	37687	7%			OORL	OORL		

Table 2: Calibration 1 Summary

Calibrator Level	Reported Value [ng/dL]	Theranos Result [ng/dL]	%CV	% Recovery
Calibrator 1	1420	1389.02	16%	98%
Calibrator 2	710	754.71	7%	106%
Calibrator 3	213	207.23	13%	97%
Calibrator 4	142	140.21	9%	99%
Calibrator 5	113.6	105.90	12%	93%
Calibrator 6	71	85.74	7%	121%
Calibrator 7	47.6	42.13	25%	89%
Calibrator 8	28.4	OORL	OORL	OORL

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Calibrator 9	0	OORL	OORL	OORL
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Figure 1: Calibration 1

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3.3 Calibration 2 (Edison 3.5, 4uL)

Of 9 tT3 levels, 8 had a back calculated mean value within $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ and ULOQ standards) of their nominal values, and all levels had CVs less than 20%. This calibration set was used to analyze Precision (4).

Table 3: Calibration Data

Reported Value [ng/dL]	All Points		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV
1420	651	589	651	589	620	7%	665	9%	1478.07	1585.54	1457.14	6%
	705	713	705	713	709	1%			1398.76	1387.94		
	130	110	DARK	DARK								
710	1303	1325	1303	1325	1314	1%	1761	27%	925.92	915.68	757.44	18%
	2454	2256	2454	2256	2355	6%			604.00	640.19		
	1653	1575	1653	1575	1614	3%			790.27	816.20		
213	5627	7413		7413			8393	11%		248.82	219.25	11%
	8746	9750	8746	9750	9248	8%			209.77	185.58		
	8139	7916	8139	7916	8028	2%			226.42	232.98		
142	11366	13043	11366	13043	12205	10%	11583	11%	153.28	125.99	149.44	15%
	11895	11981	11895	11981	11938	1%			144.09	142.65		
	7709	9628		9628						188.33		
113.6	16043	16414	16043	16414	16229	2%	16441	7%	87.75	83.73	83.44	14%
	15329	17977	15329	17977	16653	11%			95.89	68.08		
	19619			DARK								
71	12869	16896		16896			16728	6%		78.68	80.42	14%
	15319	22523	15319						96.00			
	16943	17752	16943	17752	17348	3%			78.20	70.21		
47.6	20997	20207	20997	20207	20602	3%	19622	6%	42.72	48.82	53.58	19%
	14849	15828										
	18943	18339	18943	18339	18641	2%			59.34	64.73		
28.40	25241	25954	25241	25954	25598	2%	25122	24%	OORL	OORL	OORL	OORL
	31979	5	31979	DARK					OORL			
	33862	32268	33862	32268	33065	3%			OORL	OORL		
	16572	22016	16572	22016	19294	20%			82.05	OORL		
	16599	28733	16599	28733	22666	38%			81.77	OORL		
	23392	19729	23392	19729	21561	12%			OORL	52.69		
0.00	33048	34179	33048	34179	33614	2%	27363	14%	OORL	OORL	OORL	OORL
	25608	28139	25608	28139	26874	7%			OORL	OORL		
	24981	27149	24981	27149	26065	6%			OORL	OORL		
	19430	25061	19430	25061	22246	18%			55.17	OORL		
	27892	29725	27892	29725	28809	4%			OORL	OORL		
	25527	27617	25527	27617	26572	6%			OORL	OORL		


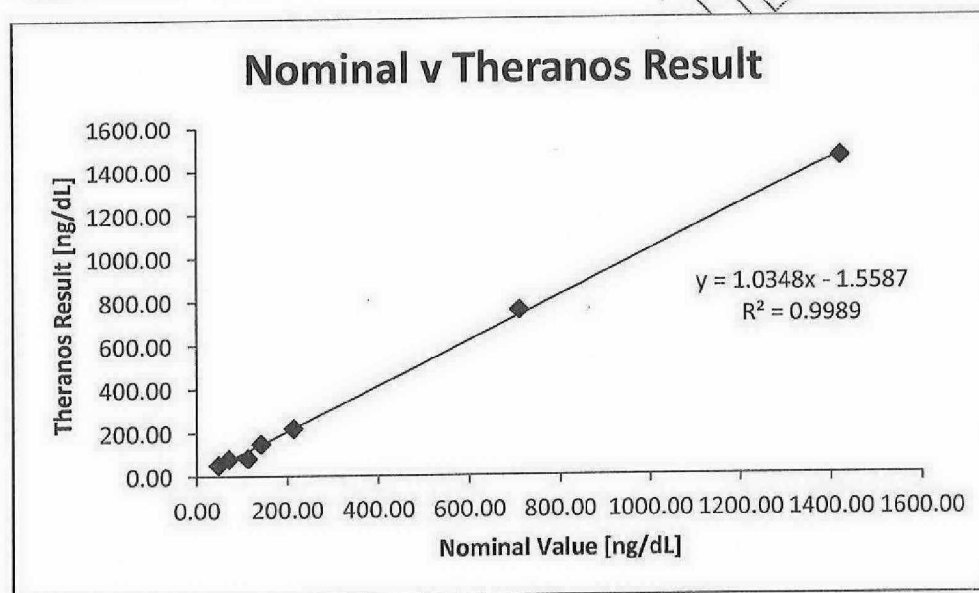

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Table 4: Calibration 2 Summary

Calibrator Level	Reported Value [ng/dL]	Theranos Result [ng/dL]	%CV	% Recovery
Calibrator 1	1420.00	1457.14	6%	103%
Calibrator 2	710.00	757.44	18%	107%
Calibrator 3	213.00	219.25	11%	103%
Calibrator 4	142.00	149.44	15%	105%
Calibrator 5	113.60	83.44	14%	73%
Calibrator 6	71.00	80.42	14%	113%
Calibrator 7	47.60	53.58	19%	113%
Calibrator 8	28.40	OORL	OORL	OORL
Calibrator 9	0.00	OORL	OORL	OORL

Figure 2: Calibration 2

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4 PRECISION

4.1 Precision was evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.

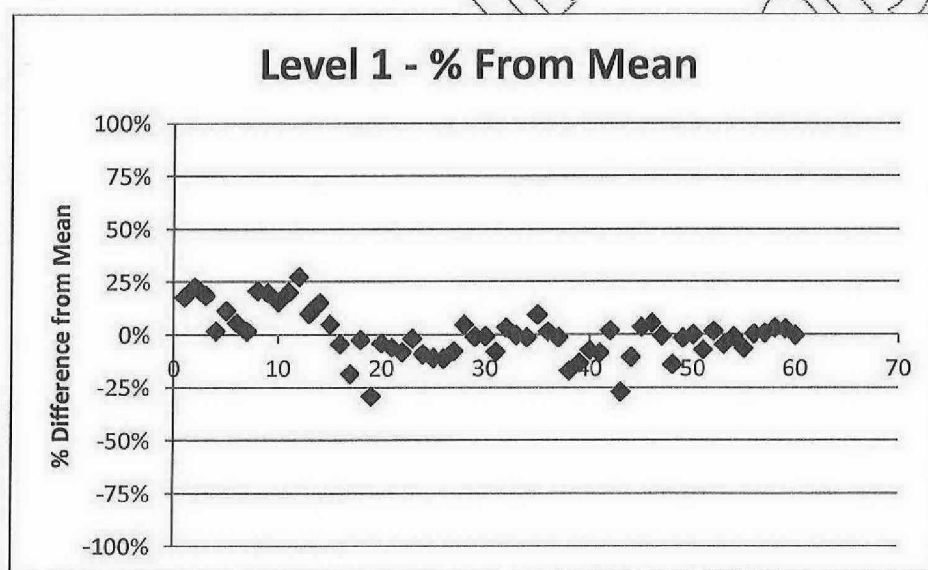
4.1.1 A total of 20 runs were performed.

4.1.2 Summary of precision results

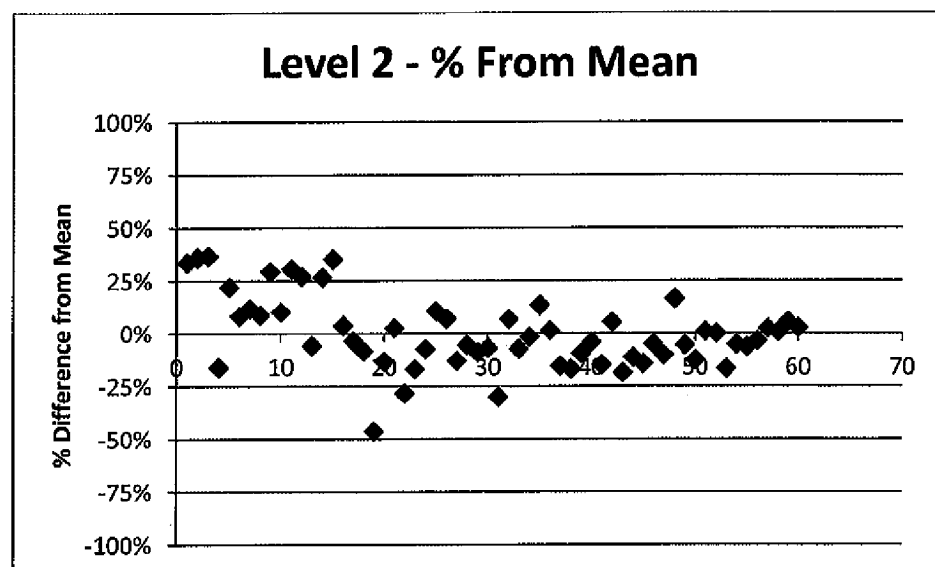
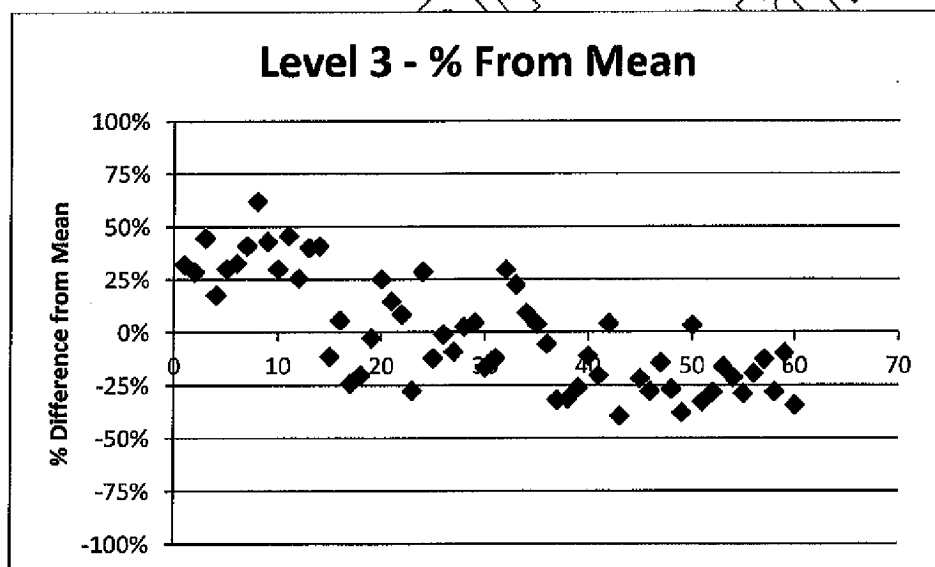
Table 5: Precision Summary

Level	Reported Value	Theranos Average	%CV
1	310	325	11%
2	109	128	17%
3	85	64	26%

Figure 3: Level 1 Precision



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Figure 4: Level 2 Precision**Figure 5: Level 3 Precision**

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5 ACCURACY/COMPARABILITY

To test the accuracy of the assay on the Theranos System, 113 unique patient samples were assayed using both the predicate method (Siemens Immulite) and the Theranos System. Of these samples 55 were within the reference range (84 – 172 ng/dL), 26 were below the reference range, and 32 were higher than the reference range. Based on the results of the data examination, either a simple linear regression or alternative procedures were used to estimate expected (average) bias and the confidence interval of expected bias at the desired medical decision level(s) as per CLSI guidance EP09-A2. StatisPro was used for bias calculations. These estimates were compared with internal criteria to judge the acceptability of the Theranos method. Each sample was run in replicate on the predicate, and the average used for comparison to the Theranos method. Some samples were stored before analysis on both methods. If the confidence interval for the predicted bias includes the defined acceptable bias or if the acceptable bias is greater than the higher limit of the confidence interval of the predicted bias, then the data do not show that the bias of the Theranos method is different from the acceptable bias or there is a high probability (97%) that the predicated bias is acceptable, respectively. The acceptable bias at each medical decision level was determined based on the total allowable error (TEa) minus the measured precision at the level closest to that decision level. Total allowable error (TEa) was taken from CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register February 28, 1992;57(40):7002-186, when available.

Each sample was tested in replicates of three. Calculated concentrations are based on the average RLU of 3 cartridges excluding outliers and dark count tips.

5.1 The analysis in the report will contain the following elements:

5.1.1 Repeatability plots, difference plots, and/or scatter plots

5.1.2 The result of an adequate range test (linear fit with r value) to ensure that the sample results span the measuring interval

5.1.3 The results of the calculations for the predicted bias (or difference) estimates and confidence intervals

5.1.4 Statement of performance claims

5.1.4.1 Default acceptance criteria for immunoassays are absolute mean bias (%RE) $\leq 20\%$ ($\leq 25\%$ at LLOQ) and total error (sum of the %CV and absolute %RE) $\leq 30\%$ ($\leq 40\%$ at the LLOQ)

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Table 6: Clinical Correlation and Bias Correction

Sample ID	Predicate Value [ng/dL]	Theranos Result [ng/dL]	% recovery
Patient 1	103	99	96%
Patient 2	86.4	119	138%
Patient 3	126	121	96%
Patient 4	155	158	102%
Patient 10	267	291	109%
Patient 19	193	209	108%
Patient 20	173	200	115%
Patient 31	83.3	88	106%
Patient 42	72.7	80	110%
Patient 60	54.2	70	130%
Patient 5	122	151	124%
Patient 6	123	120	98%
Patient 7	120	149	124%
Patient 21	176	130	74%
Patient 62	53.2	39	73%
Patient 8	130	113	87%
Patient 9	148	142	96%
Patient 11	129	104	104%
Patient 12	148	141	95%
Patient 13	129	113	87%
Patient 14	143	129	90%
Patient 15	140	111	79%
Patient 16	144	110	76%
Patient 17	134	94	70%
Patient 18	145	96	66%
Patient 22	162	139	86%
Patient 23	148	121	82%
Patient 24	160	195	122%
Patient 25	115	72	62%
Patient 26	163	143	88%
Patient 27	118	131	111%
Patient 28	118	70	60%
Patient 29	149	108	73%
Patient 30	156	135	87%
Patient 32	157	127	81%
Patient 33	125	85	68%
Patient 34	132	95	72%
Patient 35	111	78	70%
Patient 36	170	156	91%
Patient 37	102	73	72%
Patient 38	95.9	70	73%
Patient 39	172	155	90%
Patient 40	117	80	68%

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Patient 41	145	142	98%
Patient 44	133	88	66%
Patient 46	144	118	82%
Patient 47	148	173	117%
Patient 48	120	90	75%
Patient 49	103	69	67%
Patient 50	107	69	64%
Patient 51	156	112	72%
Patient 52	165	156	94%
Patient 53	98.1	107	109%
Patient 54	102	103	101%
Patient 56	103	83	81%
Patient 57	130	167	128%
Patient 58	167	177	106%
Patient 66	128	137	107%
Patient 69	163	132	81%
Patient 74	157	184	117%
Patient 77	151	131	87%
Patient 84	163	138	84%
Patient 43	178	109	61%
Patient 45	213	202	95%
Patient 55	184	174	94%
Patient 59	182	174	95%
Patient 61	238	249	105%
Patient 63	189	185	98%
Patient 64	189	165	87%
Patient 65	206	203	99%
Patient 68	205	249	122%
Patient 70	266	304	114%
Patient 71	209	188	90%
Patient 72	202	179	89%
Patient 73	187	249	133%
Patient 75	258	324	125%
Patient 78	238	268	113%
Patient 80	213	200	94%
Patient 81	238	286	120%
Patient 82	196	179	91%
Patient 83	205	236	115%
Patient 88	244	189	77%
Patient 89	224	253	113%
Patient 91	192	214	111%
Patient 92	255	333	130%
Patient 93	226	159	71%
Patient 95	181	218	121%
Patient 97	241	237	98%
Patient 98	237	245	104%

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Patient 100	193	204	106%
Patient 67	55.6	68	123%
Patient 76	73.5	59	80%
Patient 79	52.9	OORL	
Patient 85	48.5	43	88%
Patient 86	47.9	OORL	
Patient 87	51.2	OORL	
Patient 90	45.7	41	90%
Patient 94	74.1	88	119%
Patient 96	70.6	70	100%
Patient 99	47	OORL	
Patient FT3 16	43.8	41	93%
Patient FT3 29	82.8	81	97%
Patient FT3 37	48.9	40	82%
Patient FT3 39	42.8	OORL	
Patient FT3 50	66.7	61	92%
Patient FT3 52	58	57	97%
Patient FT3 54	44.2	OORL	
Patient FT3 57	77.7	64	82%
Patient FT3 58	78.5	81	103%
Patient FT3 61	53.1	47	89%
Patient FT3 75	145	122	84%
Patient FT3 94	61.1	67	109%
Patient FT3 98	58.9	75	127%


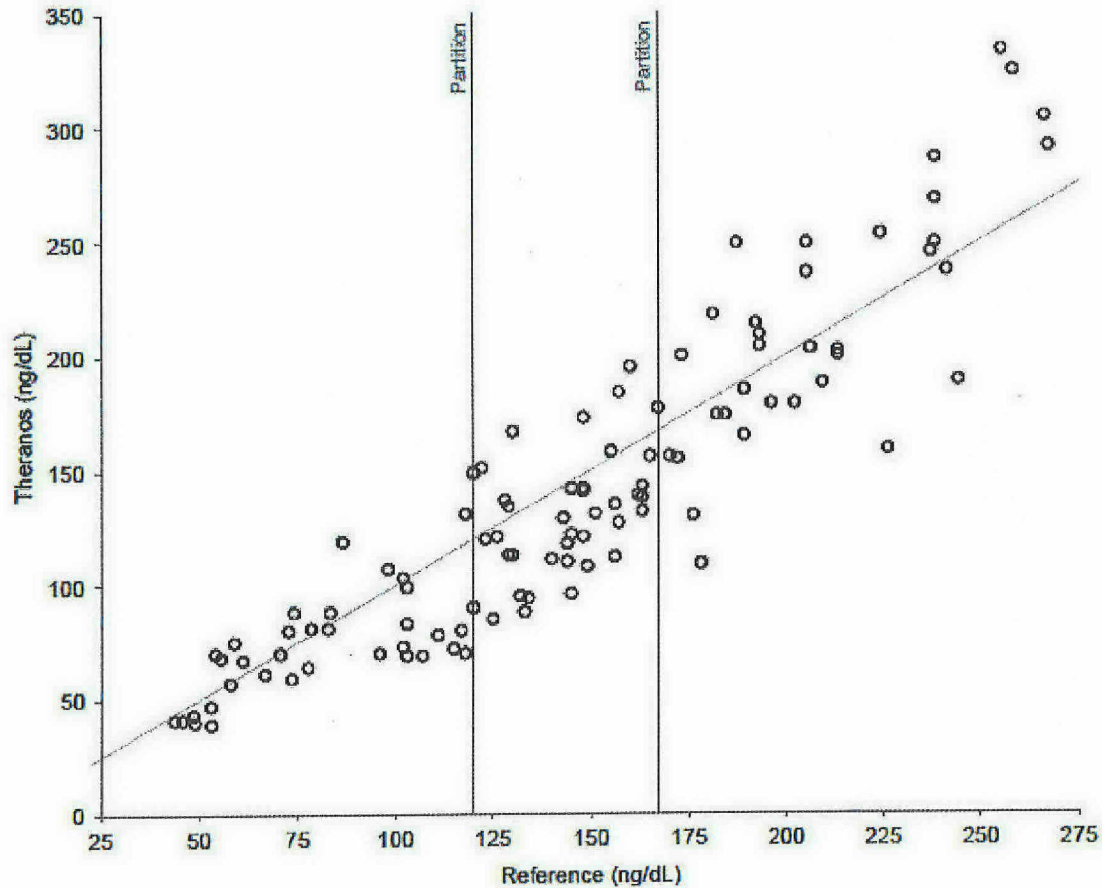
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Figure 6: Scatter Plot – CLSI Guideline EP09-A2-IR Section 4.2

Scatter plot*CLSI guideline EP09-A2-IR section 4.2*


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Figure 7: Difference Plot – CLSI Guideline EP09-A2-IR Section 4.2

Difference plot

CLSI guideline EP09-A2-IR section 4.2

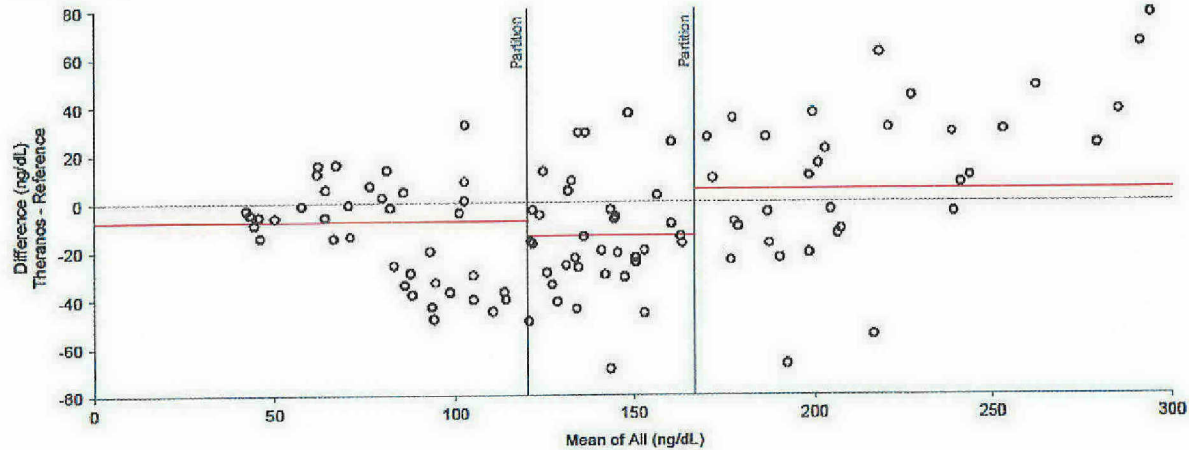


Table 7: Adequate Range Test – CLSI Guideline EP09-A2-IR Section 4.5

Adequate range test

CLSI guideline EP09-A2-IR section 4.5

r 0.914

$r < 0.975$ indicates that the error in X is not adequately compensated by the measuring range. CLSI recommends use of partitioned biases.

Table 8: Partitioned Differences – CLSI Guideline EP09-A2-IR Section 6.2

Partitioned differences

CLSI guideline EP09-A2-IR section 6.2

Partition	n	Mean difference	SD
< 120	34	-7.57	19.30
≥ 120 and < 167	38	-13.58	24.04
≥ 167	35	5.31	35.44


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Table 9: Comparability – CLSI Guideline EP09-A2-IR Section 7**Comparability**

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
1	80.0	-7.57	3.310	-14.30 to -0.83	8.80
2	100.0	-7.57	3.310	-14.30 to -0.83	11.00
3	350.0	5.31	5.991	-6.86 to 17.49	38.50

Difference is less than allowable bias: 11%.

Table 10 Method comparison summary

Method Comparison			
Levels (ng/dL)	Precision (%)	Total Error (%)	Allowable Bias (%)
80	24	35	11
100	17	35	18
350	15	35	20

6 DILUTION LINEARITY

A high analyte sample was selected and serially diluted using a patient sample with undetectable levels of tT3 to a total of 10 points to test the linearity. The nominal values of each level were calculated based on the value of the high analyte sample as reported by the predicate method (Siemens Immulite).

Each dilution level was tested on the Theranos System and compared to the nominal concentrations. For each dilution level, the recovery should be within $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ) of their nominal value, and when plotted, the R^2 value should be equal or greater than 0.95.

Table 11: Dilution Linearity

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			%CV
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	
266	6384	6752	6384	6752	6568	4%	6408	18%	304.83	290.37	303.87	17%
	7049	7920	7049	7920	7485	8%			279.59	251.74		
	5679	4661	5679	4661	5170	14%			336.69	396.24		
138	13522	14634	13522	14634	14078	6%	12903	13%	144.30	130.74	152.59	15%
	1044	989										

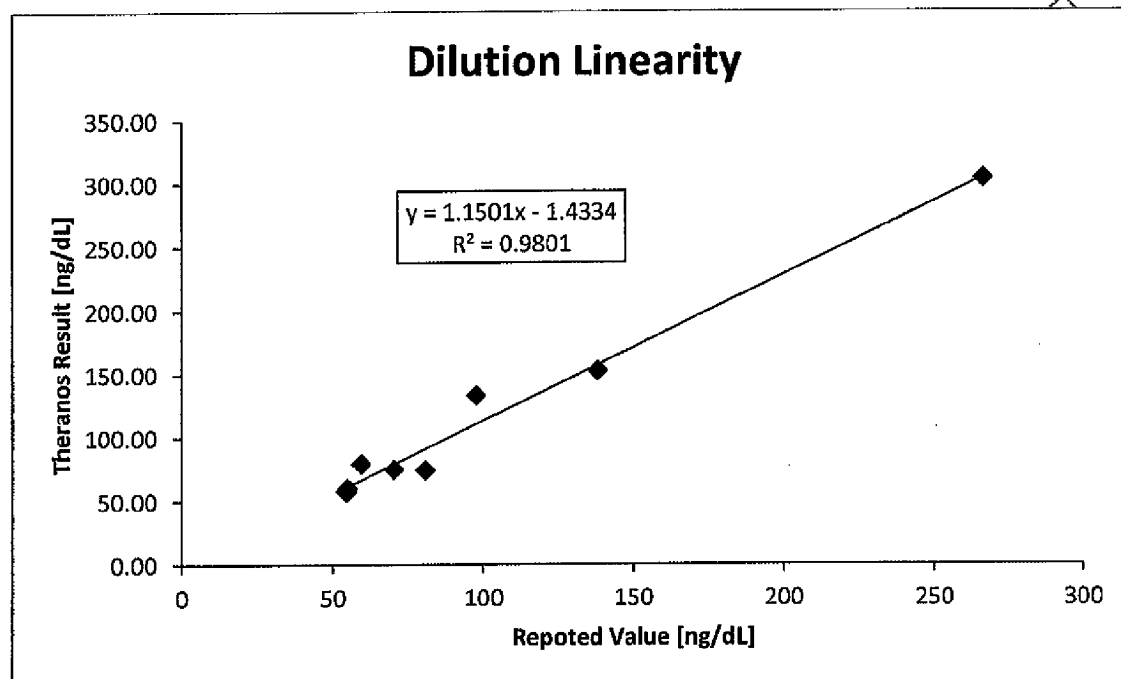
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	12662	10792	12662	10792	11727	11%			155.97	185.98		
97.6	13658	12165	13658	12165	12912	8%	14409	17%	142.56	163.27	133.37	20%
	12293	13564	12293	13564	12929	7%			161.35	143.76		
	17691	17081	17691	17081	17386	2%			100.14	105.69		
	20234	20239	20234	20239	20237	0%			79.95	79.92		
80.6	23692	25993	23692	25993	24843	7%	21031	15%	57.62	45.11	74.35	29%
	17748	18280	17748	18280	18014	2%			99.64	95.11		
	20652	22082	20652	22082	21367	5%			76.98	67.41		
70.1	20723	19494	20723	19494	20109	4%	20933	19%	76.48	85.44	75.02	20%
	18185	24464	18185	24464	21325	21%			95.90	53.24		
	21150	25152	21150	25152	23151	12%			73.54	49.50		
59.3	20241	14706	20241	14706	17474	22%	20312	21%	79.90	129.92	79.39	43%
	11239	11020										
	21576	22097	21576	22097	21837	2%			70.69	67.31		
54.2	42816	41838					23716	10%			57.48	23%
	24792	26400	24792	26400	25596	4%			51.44	43.05		
	17504	17921	17504	17921	18713	2%			101.78	98.15		
54.8	34855	2	34855	DARK			23229	30%	OORL		60.34	36%
	23252	22612	23252	22612	22932	2%			60.20	64.08		
	20683	25204	20683	25204	22944	14%			76.76	49.22		
54.7	21239	25549	21239	25549	23394	13%	23730	9%	72.94	47.40	57.40	23%
	24386	26319	24386	25316	24851	3%			53.68	48.63		
	45037	42845	45037	42845	43941	4%			OORL	OORL		
<40ng/dL	35262	40590	35262	40590	37926	10%	41011	8%	OORL	OORL	OORL	OORL
	41063	41267	41063	41267	41165	0%			OORL	OORL		

Table 12: Dilution Linearity Summary

Sample ID	Predicate Value [ng/dL]	Theranos Result [ng/dL]	% Recovery to CLIA
High Patient Sample #70	266	303.87	114%
1/2	138	152.59	111%
1/3	97.6	133.37	137%
1/4	80.6	74.35	92%
1/5	70.1	75.02	107%
1/6	59.3	79.39	134%
1/7	54.2	57.48	106%
1/8	54.8	60.34	110%
1/9	54.7	57.40	105%
low sample	<40	OORL	Na

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Figure 8: Dilution Linearity

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7 REFERENCE RANGE

To verify the accuracy of the Theranos System, the reference range was tested and compared to the results from the predicate method. According to C.F.R. Ch IV, § 493.1253 "Standard: Establishment and verification of performance specifications" and outlined in CLSI guideline C28A3, laboratories developing test methods need to verify the measuring interval. Additionally, by verifying the measuring interval, clinical laboratories can ensure that the calibration of the measurement procedure is correctly applicable over the range in which they report patient results and that the measuring interval they are obtaining in their laboratory is comparable to the interval defined by the manufacturer in the product insert. A measuring interval consists of all numeric values between the lower and upper numeric values for which a method can produce quantitative results suitable for clinical use.

7.1 Calculated concentrations are based on the mean RLU of 3 cartridges tested.

7.2 Acceptance Criteria: In accordance to CLSI guideline C28-A3c,

7.2.1 95% of at least 20 samples tested must be within the reference interval.

The reference interval established for the predicate method is 84 – 172 ng/dL.

Of 20 unique samples collected from reference donors, zero patients had tT3 levels outside of the reference range. These samples were confirmed via predicate method listed in section 1.2.

Overall, 100% of the patients tested fell within the reference range, and is acceptable for verification of the reference range.

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Table 13: Normal Patient Sample

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			%CV
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip1	Tip2	Mean	
103	16166	19496	16166	19496	17831	13%	16140	18%	114.35	85.42	114.62	27%
	16666	18601	16666	18601	17634	8%			109.49	92.46		
	11630	14280	11630	14280	12955	14%			171.64	134.89		
98.4	31994	28802					17886	13%	123.40	109.35	98.45	21%
	15293	16680	15293	16680	15987	6%			89.14	77.68		
	19016	20553	19016	20553	19785	5%						
106	14882	16234	14882	16234	15558	6%	16981	17%	127.92	113.68	106.53	23%
	19850	21140	19850	21140	20495	4%			82.76	73.61		
	14424	15357	14424	15357	14891	4%			133.19	122.71		
85.5	16411	17161	16411	17161	16786	3%	16407	5%	111.94	104.87	111.98	7%
	11	15649	DARK	15649						119.62		
	443	49		DARK								
115	12801	11436	12801	11436	12119	8%	14344	17%	154.01	174.82	134.13	21%
	14104	13329	14104	13329	13717	4%			137.01	146.82		
	15970	18424	15970	18424	17197	10%			116.32	93.91		
89.7	16775	17104	16775	17104	16940	1%	16182	7%	108.45	105.39	114.19	10%
	10283	12498										
	16180	14670	16180	14670	15425	7%			114.22	130.33		
109	21312	22195	21312	22195	21754	3%	18905	19%	72.45	66.69	90.02	37%
	15506	13282	15506	13282	14394	11%			121.13	147.45		
	21412	19724	21412	19724	20568	6%			71.78	83.70		
99.7	13861	16815	13861	16815	15338	14%	17181	18%	140.00	108.08	104.69	28%
	13515	18272	13515	18272	15894	21%			144.39	95.18		
	20221	20404	20221	20404	20313	1%			80.05	78.73		
97	18686	17207	18686	17207	17947	6%	16782	10%	91.78	104.45	108.39	15%
	1	4	DARK	DARK								
	14549	16684	14549	16684	15617	10%			131.73	109.32		
114	13605	14626	13605	14626	14116	5%	16192	19%	143.23	130.84	114.09	24%
	21319	18334	21319	18334	19827	11%			72.40	94.66		
	14730	14539	14730	14539	14635	1%			129.64	131.84		
114	11303	13063	11303	13063	12183	10%	14080	18%	177.04	150.39	137.30	23%
	15898	16683	15898	16683	16291	3%			117.05	109.33		
	11206	16329	11206	16329	13768	26%			178.69	112.74		
94.7	18926	21163	18926	21163	20045	8%	19194	7%	89.85	73.46	87.75	11%
	18181	18507	18181	18507	18344	1%			95.94	93.23		
	24007	26095										
110	15843	15532	15843	15532	15688	1%	19159	17%	117.62	120.85	88.02	30%
	20946	20502	20946	20502	20724	2%			74.94	78.04		
	22974	24238	22974						61.87			
99.9	14922	17845	14922	17845	16384	13%	17212	10%	127.48	98.80	104.41	15%

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	15951	16452	15951	16452	16202	2%			116.52	111.54		
	18241	19861	18241	19861	19051	6%			95.43	82.68		
103	16230	16386	16230	16386	16308	1%	16105	12%	113.72	112.19	114.97	18%
	16278	12726	16278	12726	14502	17%			113.25	135.06		
	16040	18968	16040	18968	17504	12%			115.62	89.52		
	13409	16633	13409	16633	15021	15%			145.77	109.80		
103	16818	14180	16818	14180	15499	12%	15933	11%	108.05	136.09	116.70	16%
	17009	17549	17009	17549	17279	2%			106.27	101.38		
	20081	20420	20081	20420	20251	1%			81.06	78.62		
94.2	14896	17600	14896	17600	16248	12%	17087	16%	127.77	100.93	105.55	23%
	15269	14254	15269	14254	14762	5%			123.66	135.20		
	21061	22116										
98	15579	12728	15579	12728	14154	14%	14830	10%	120.36	155.03	128.52	14%
	14875	16136	14875	16136	15506	6%			128.00	114.65		
	16637	17078	16637	17078	16858	2%			109.76	105.63		
108	21198	19538	21198	19538	20368	6%	19166	10%	73.22	85.10	87.97	18%
	21071	19471	21071	19471	20276	6%			74.08	85.61		
	18825	18813	18825	18813	18819	0%			90.66	90.75		
85.9	10061	10900					17104	12%			105.40	18%
	15739	15037	15739	15037	15388	3%			118.69	126.20		

Table 14: Reference Range Summary

Sample ID	Reported Value [ng/dL]	Theranos Result [ng/dL]
Patient 1	103.00	114.62
Patient 2 (4P)	98.40	98.45
Patient 3	106.00	106.53
Patient 4 (5P)	85.50	111.98
Patient 5	113.00	134.13
Patient 6	89.70	114.19
Patient 7 (14P)	109.00	90.02
Patient 8	99.70	104.69
Patient 9	97.00	108.39
Patient 10	114.00	114.09
Patient 11	114.00	137.30
Patient 12	94.70	87.75
Patient 13	110.00	88.02
Patient 14	99.90	104.41
Patient 15 (15P)	103.00	114.97
Patient 16	103.00	116.70
Patient 17	94.20	105.55
Patient 18	98.00	105.59
Patient 19	108.00	87.97
Patient 20	85.90	105.40

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8 BLOOD COLLECTION DEVICE (BCD) COMPARISON

Because the sample volume obtained from a fingerstick is sufficient only for testing on the Theranos System but not in the predicate method, a second verification of the reference range was performed using matched fingerstick blood and venous blood.

8.1 Calculated concentrations are based on the mean RLU of 3 cartridges tested.

8.2 Acceptance Criteria: In accordance to CLSI guideline C28-A3c,

8.2.1 95% of at least 20 samples tested must be within the reference interval.

The reference interval established for the predicate method is 84 – 172 ng/dL.

19 out of 20 venous blood sample collected from unique donors were within reference range, 100% of BCD sample collected from these donors had T3 levels within the reference range.


Overall, 100% of the patients tested for BCD fell within the reference range, and is acceptable for verification of the reference range.

Table 15: Venous v. Fingerstick

Sample ID	Sample Type	All-Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			
		Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV
Patient 1	Venous Tube 1	17815	20108	17815		17815		15808	17%	99.06		120.41	25%
	Venous Tube 2	12793	16817	12793	16817	14805	19%			154.12	108.06		
	Fingerstick 1	11706	12891	11706	12891	12299	7%	13018	8%	170.41	152.75	151.59	9%
	Fingerstick 2	13476	13998	13476	13998	13737	3%			144.90	138.30		
Patient 2	Venous Tube 1	13482	13388	13482	13388	13435	0%	14669	18%	144.82	146.05	133.11	21%
	Venous Tube 2	13085	18720	13085	18720	15903	25%			150.09	91.50		
	Fingerstick 1	18845	21413	18845		18845		15583	19%	90.50		123.05	24%
	Fingerstick 2	13127	14776	13127	14776	13952	8%			149.52	129.12		
Patient 3	Venous Tube 1	10840	11711	10840	11711	11276	5%	12911	15%	185.11	170.33	154.84	18%
	Venous Tube 2	13979	15113	13979	15113	14546	6%			138.54	125.36		
	Fingerstick 1	12473	12810	12473	12810	12642	2%	13924	16%	158.69	153.88	141.25	19%
	Fingerstick 2	78345	16490		16490	16490					111.18		
Patient 4	Venous Tube 1	14902	69	14902	DARK	14902		12303	18%	127.70		164.06	19%
	Venous Tube 2	10853	11153	10853	11153	11003	2%			184.88	179.60		
	Fingerstick 1	19526	21239	19526		19526		15296	24%	85.20		127.65	29%
	Fingerstick 2	12824	13539	12824	13539	13182	4%			153.68	144.08		
Patient 5	Venous Tube 1	17528	17917	17528	17917	17723	2%	18116	3%	101.57	98.18	96.57	5%
	Venous Tube 2	18092	18925	18092	18925	18509	3%			96.69	89.86		
	Fingerstick 1	14704	20361	14704	20361	17533	23%	17777	13%	129.94	79.04	100.89	21%
	Fingerstick 2	18125	17918	18125	17918	18022	1%			96.41	98.17		
Patient 6	Venous Tube 1	13455	10578	13455	10578	12017	17%	12328	10%	145.17	189.92	161.99	12%

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	Venous Tube 2	12217	13062	12217	13062	12640	5%			162.48	150.40		
	Fingerstick 1	12465	15879	12465	15879	14172	17%	14328	10%	158.81	117.25	135.39	14%
	Fingerstick 2	13829	15138	13829	15138	14484	6%			140.40	125.09		
Patient 7	Venous Tube 1	12874	13303	12874	13303	13089	2%	14054	12%	152.99	147.17	138.77	14%
	Venous Tube 2	76945	15986		15986	15986				116.16	64.82		
	Fingerstick 1	114	18375	DARK	18375	18375		18759	5%	97.17	82.63	91.37	8%
Patient ft1	Fingerstick 2	18035	19868	18035	19868	18952	7%						
	Venous Tube 1	19998	16566		16566	16566		18357	8%		110.44	95.08	15%
	Venous Tube 2	18627	19878	18627	19878	19253	5%			92.25	82.56		
Patient 9	Fingerstick 1	15330	19969	15330	19969	17650	19%	16283	18%	123.00	81.88	115.84	24%
	Fingerstick 2	13164	16668	13164	16668	14916	17%			149.02	109.47		
	Venous Tube 1	18887	20404	18887	20404	19646	5%	18752	7%	90.16	78.73	91.59	11%
Patient 10	Venous Tube 2	18227	17490	18227	17490	17859	8%			95.55	101.91		
	Fingerstick 1	16205	18168	16205	18168	17187	8%	26053	40%	113.97	96.05	105.01	12%
	Fingerstick 2	33455	36382	33455	36382					OORL	OORL		
Patient 10	Venous Tube 1	16223	19369	16223	19369	17796	13%	15817	24%	113.79	86.39	122.72	34%
	Venous Tube 2	10544	11859		11859	11859					167.99		
	Fingerstick 1	15880	17068	15880	17068	18474	5%	17502	7%	117.24	105.72	102.27	11%
Patient ft10	Fingerstick 2	18429	18629	18429	18629	18529	1%			93.87	92.24		
	Venous Tube 1	10807	12391	10807	12391	11599	10%	11321	7%	185.71	159.90	173.87	7%
	Venous Tube 2	11364	9997	11364		11364				176.02			
Patient ft14	Fingerstick 1	10882	11386	10882	11386	11134	3%	12720	22%	184.36	175.65	159.04	23%
	Fingerstick 2	15892	17210	15892		15892				117.12			
	Venous Tube 1	15812	14614	15812	14614	15243	6%	18863	23%	117.94	130.97	94.58	37%
Patient 13	Venous Tube 2	22711	22316	22711	22316	22514	1%			63.47	65.93		
	Fingerstick 1	18525	18735	18525	19735	19130	4%	18661	6%	93.08	83.62	92.27	10%
	Fingerstick 2	17189	19201	17183	19201	18192	8%			104.67	87.69		
Patient 13	Venous Tube 1	15998	15687	15998	15687	15843	1%	14975	10%	116.04	119.23	127.91	14%
	Venous Tube 2	15492	12722	15492	12722	14107	14%			121.27	155.12		
	Fingerstick 1	10027	14887	10027	14887	12457	28%	13849	18%	200.63	127.87	144.11	26%
Patient ft4	Fingerstick 2	15307	15175	15307	15175	15241	1%			123.25	124.68		
	Venous Tube 1	19468	19208	19468	19208	19338	1%	19591	10%	85.64	87.64	85.53	18%
	Venous Tube 2	17389	22298	17389	22298	19844	17%			102.81	66.04		
Patient 15	Fingerstick 1	24202	25064	24202	25064	24633	2%	20846	21%	54.71		88.90	34%
	Fingerstick 2	17743	16373	17743	16373	17058	6%			99.68	112.31		
	Venous Tube 1	30327	30956					15259	1%			123.78	1%
Patient 16	Venous Tube 2	15148	15369	15148	15369	15259	1%			124.98	122.58		
	Fingerstick 1	18389	17261	18389	17261	17825	4%	17981	11%	94.20	103.96	98.67	17%
	Fingerstick 2	20545	15728	20545	15728	18137	19%			77.73	118.80		
Patient 16	Venous Tube 1	17756	20346	17756	20346	19051	10%	17095	15%	99.57	79.15	107.43	22%
	Venous Tube 2	14249	16028	14249	16028	15139	8%			135.26	115.74		
	Fingerstick 1	19030	21102	19030	21102	20066	7%	17395	18%	89.03	73.87	105.61	27%
Patient ft5	Fingerstick 2	15029	14417	15029	14417	14723	3%			126.29	133.27		
	Venous Tube 1	12190	13371	12190	13371	12781	7%	13445	20%	162.89	146.27	148.78	22%
	Venous Tube 2	11077	17142	11077	17142	14110	30%			180.92	105.04		
	Fingerstick 1	13912	15066	13912	15066	14489	6%	12633	19%	139.36	125.88	160.21	23%

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	Fingerstick 2	9675	11880	9675	11880	10778	14%			207.96	167.66		
Patient ft13	Venous Tube 1	16424	16062	16424	16062	16243	2%	18586	15%	111.82	115.39	94.47	24%
	Venous Tube 2	22036	19823	22036	19823	20930	7%			67.70	82.96		
	Fingerstick 1	15058	19340	15058	19340	17199	18%	17199	18%	125.96	86.62	106.29	26%
	Fingerstick 2	23687	24636										
Patient 19	Venous Tube 1	14550	13641	14550	13641	14096	5%	17918	26%	131.71	142.77	103.80	39%
	Venous Tube 2	19869	23613	19869	23613	21741	12%			82.62	58.08		
	Fingerstick 1	18813	20869	18813	20869	19841	7%	18355	11%	90.35	75.47	95.39	17%
	Fingerstick 2	16384	17353	16384	17353	16869	4%			112.21	103.13		
Patient ft16	Venous Tube 1	15422	19794	15422	19794	17608	18%	17126	12%	122.02	83.18	106.44	18%
	Venous Tube 2	15463	17826	15463	17826	16645	10%			121.58	98.96		
	Fingerstick 1	13278	14748	13278	14748	14013	7%	14251	9%	147.50	129.44	136.02	11%
	Fingerstick 2	13105	15871	13105	15871	14488	18%			149.82	117.33		

Table 16: Venous v. Fingerstick Comparison Summary

Sample ID	Venous Tube	Fingerstick
	Original	Original
Patient 1	120.41	151.59
Patient 2	133.11	123.05
Patient 3	154.84	141.25
Patient 4	164.06	127.65
Patient 5	96.57	100.89
Patient 6	161.99	135.39
Patient 7	138.77	91.37
Patient ft1	95.08	115.84
Patient 9	91.59	105.01
Patient 10	122.72	102.27
Patient ft10	173.87	159.04
Patient ft14	94.58	92.27
Patient 13	127.91	144.11
Patient ft4	85.53	88.90
Patient 15	123.78	98.67
Patient 16	107.43	105.61
Patient ft5	148.78	160.21
Patient ft13	94.47	106.29
Patient 19	103.80	95.39
Patient ft16	106.44	136.02

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9 ANALYTICAL SENSITIVITY

In accordance to CLSI guideline EP17-A2 for analytical sensitivity verification, 60 replicates of a blank sample and 60 replicates of the LLOQ were tested.

9.1 Verification of Limit of Blank

- 9.1.1 Acceptance Criteria: the percentage of measured blanks of the total blanks replicates should be equal or greater than 90%
- 9.1.2 When the results are calculated by cartridge, for a total of 60 replicates, 100% replicates returned OORL results. By grouping the results into 20 replicates of 3 cartridges, 100% returned OORL results.

9.2 Verification of Limit of Detection

- 9.2.1 Acceptance Criteria: the percentage of measureable results to the total LLOQ replicates should be equal or greater than 90%
- 9.2.2 When the results are calculated by cartridge, for a total of 56 replicates, 91% replicates returned quantifiable results. By grouping the results into 20 replicates of 3 cartridges, 90% returned quantifiable results.

The LLOQ sample was diluted 1:1 and 20 replicates of the 1/2xLLOQ sample were tested. Zero replicates were within the assay reportable range.

- 9.3 Of 20 replicates (calculated by cartridge) at 1/2xLLOQ, 0% returned quantifiable results

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Table 17: 1/2 LLOQ

All Tips		Intra-Cartridge		
Tip1	Tip2	Mean	%CV	Result
39219	44045	41632	8%	OORL
28547	32034	30291	8%	OORL
33571	39866	36719	12%	OORL
30675	32430	31553	4%	OORL
23990	31556	27773	19%	OORL
39071	47623	43347	14%	OORL
51459	52834	52147	2%	OORL
30242	34403	32323	9%	OORL
34958	38776	36867	7%	OORL
35964	41491	38728	10%	OORL
31981	35383	33682	7%	OORL
43912	44876	44394	2%	OORL
41150	40804	40977	1%	OORL
39189	50810	45000	18%	OORL
33147	34652	33900	3%	OORL
31669	31532	31601	0%	OORL
30900	40237	35569	19%	OORL
28654	26459	27557	6%	OORL
37580	39337	38459	3%	OORL
62870	51818	57344	14%	OORL

Table 18: Blank Replicates

Blank							
All Tips		Intra-Cartridge			Inter-Cartridge		
Tip1	Tip2	Mean	%CV	Result	Mean	%CV	Result
37691	37106	37399	1%	OORL	42652	17%	OORL
45166	56514	50840	16%	OORL			
39423	40011	39717	1%	OORL			
38977	42837	40907	7%	OORL	33120	19%	OORL
30523	30499	30511	0%	OORL			
27772	28111	27942	1%	OORL			
44933	46567	45750	3%	OORL	50681	19%	OORL
40566	45943	43255	9%	OORL			
61031	65043	63037	5%	OORL			
43093	41111	42102	3%	OORL	43123	6%	OORL
41284	40797	41041	1%	OORL			
45537	46914	46226	2%	OORL			
43330	45080	44205	3%	OORL	40203	12%	OORL
31426	42785	37106	22%	OORL			
40308	38289	39299	4%	OORL			
48424	58216	53320	13%	OORL	46258	24%	OORL

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49252	47736	48494	2%	OORL			
27664	9	27664		OORL			
62453	61022	61738	2%	OORL			
49485	55304	52395	8%	OORL	51016	20%	OORL
38498	39336	38917	2%	OORL			
61739	59807	60773	2%	OORL			
49675	51233	50454	2%	OORL	47781	27%	OORL
29482	34747	32115	12%	OORL			
40772	40083	40428	1%	OORL			
40997	41508	41253	1%	OORL	45953	17%	OORL
55635	56722	56179	1%	OORL			
37988	35837	36913	4%	OORL			
33540	40533	37037	13%	OORL	36691	8%	OORL
33841	38406	36124	9%	OORL			
61504	44120	52812	23%	OORL			
46059	46707	46383	1%	OORL	51155	13%	OORL
54049	54488	54269	1%	OORL			
55051	56616	55834	2%	OORL			
50294	53030	51662	4%	OORL	54830	5%	OORL
55500	58491	56996	4%	OORL			
42359	43729	43044	2%	OORL	51228	20%	OORL
58178	68811	63495	12%	OORL			
46890	47401	47146	1%	OORL			
45645	48323	46984	4%	OORL			
47478	41779	44629	9%	OORL	52123	19%	OORL
64031	65480	64756	2%	OORL			
59778	59967	59873	0%	OORL			
45871	54356	50113	12%	OORL	50043	20%	OORL
33262	47026	40144	24%	OORL			
36497	38260	37379	3%	OORL			
50977	56140	53559	7%	OORL	51142	23%	OORL
62461	62518	62490	0%	OORL			
31765	37540	34653	12%	OORL			
29559	33734	31647	9%	OORL	41020	30%	OORL
55649	57872	56761	3%	OORL			
42901	41793	42347	2%	OORL			
50731	50457	50594	0%	OORL	44028	12%	OORL
40003	38282	39143	3%	OORL			
43273	41652	42463	3%	OORL			
43372	47114	45243	6%	OORL	39887	16%	OORL
29776	34136	31956	10%	OORL			
39107	35765	37436	6%	OORL			
46676	46979	46828	0%	OORL	42514	13%	OORL
38409	48149	43279	16%	OORL			

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Table 19: LLOQ Replicates

All Tips		Intra-Cartridge			Inter-Cartridge		
Tip1	Tip2	Mean	%CV	Result	Mean	%CV	Result
20426	17770	19098	10%	88.50	24464	22%	53.24
22403	25030	23717	8%	57.48			
30924	30232	30578	2%	OORL			
21560	23774	22667	7%	63.74	24593	9%	52.53
24565	27616	26091	8%	44.61			
23546	26494	25020	8%	50.21			
8387	44829				18368	19%	94.38
13816	17771	15794	18%	118.13			
19995	21890	20943	6%	74.96			
24136	24230	24183	0%	34.81	28749	22%	OORL
28901	22341	25621	18%	47.03			
36624	36263	36444	1%	OORL			
22707	23685	23196	3%	60.53	24215	11%	54.64
20102	24598	22350	14%	65.71			
26219	27976	27098	8%	39.63			
23951	26707	25329	8%	48.56	23909	16%	55.86
23599	16860	20230	24%	79.99			
24652	28223	26438	10%	42.87			
25296	24410	24853	3%	51.11	25379	4%	48.30
27250	25686	26408	4%	42.71			
24520	25110	24815	2%	51.32			
25464	24019	24742	4%	51.72	24033	14%	55.66
18643	27815	23229	28%	60.34			
21935	26324	24128	13%	55.12			
20758	20957	20858	1%	75.55	25149	20%	49.51
27099	33657	30378	15%	OORL			
22113	26312	24213	12%	54.65			
20041	23212	21627	10%	70.36	27691	33%	OORL
39180	39267	39224	0%	OORL			
20259	24187	22223	12%	66.51			
20141	22243	21192	7%	73.26	23321	11%	59.79
20941	26049	23495	15%	58.77			
23909	26643	25276	8%	48.84			
21598	22102	21850	2%	68.90	21954	7%	68.23
20199	23915	22057	12%	67.57			
15922	36517						
33075	26705	29890	15%	OORL	24878	23%	50.97
814	24551	24551		52.76			
22930	17130	20030	20%	81.44			
20390	16453	18422	15%	93.93	20070	29%	81.15
16153	19170	17662	12%	100.39			
31310	16943	24127	42%	55.13			

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23096	21602	22349	5%	65.72	20721	20%	76.50
20007	20788	20398	3%	78.78			
13120	25711	19416	46%	86.04			
21489	25373	23431	12%	59.14	21226	21%	73.03
6981	23813	23813		56.92			
13988	21469	17729	30%	99.81			
336	31468				17644	7%	100.55
18947	39922	18947		89.69			
17309	16675	16992	3%	106.43			
16849	18348	17599	6%	100.95	16368	7%	112.36
15333	15207	15270	1%	123.65			
16614	15859	16237	3%	113.66			
30701	883				17618	19%	100.78
17717	13247	15482	20%	121.38			
21361	18145	19753	12%	83.49			
15961	16169	16065	1%	115.36	15131	22%	125.17
175	11431	11431		174.90			
12225	19867	16046	34%	115.56			

Table 20: Analytical Sensitivity Summary

Blank Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% OORL	Mean	%CV
Intra-Cartridge	60	45617	21%	OORL	100%	NA	NA
Inter-Cartridge	20	45772	13%	OORL	100%	NA	NA

LLOQ Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% Quantifiable	Mean	%CV
Intra-Cartridge	56	22519	22%	64.66	91%	74.68	37%
Inter-Cartridge	20	22278	17%	66.16	90%	72.92	33%

1/2 LLOQ Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% Quantifiable	Mean	%CV
Intra-Cartridge	20	37993	20%	OORL	0%	NA	NA

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10 ANTICOAGULANT COMPARISON

To test the effect of anticoagulants, 20 unique patients donated a total of 3 tubes, one EDTA, one Li-Heparin, and one Serum. Percentage of recovery compared to CLIA reported EDTA plasma sample value was used to compare anticoagulant effect. The average recoveries were within 20% of reported value. It indicated all three matrices performed equivalently in the Theranos Total T3 assay.

Table 21: EDTA Plasma

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			%CV
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	
103	16166	19496	16166	19496	17831	13%	16140	18%	114.35	85.42	114.62	27%
	16666	18601	16666	18601	17634	8%			109.49	92.46		
	11630	14280	11630	14280	12955	14%			171.64	134.89		
98.4	31994	28802					17886	13%			98.45	21%
	15293	16680	15293	16680	15987	6%			123.40	109.35		
	19016	20553	19016	20553	19785	5%			89.14	77.68		
106	14882	16234	14882	16234	15558	6%	16981	17%	127.92	113.68	106.53	23%
	19850	21140	19850	21140	20495	4%			82.76	73.61		
	14424	15357	14424	15357	14891	4%			133.19	122.71		
85.5	16411	17161	16411	17161	16786	3%	16407	5%	111.94	104.87	111.98	7%
	11	15649	DARK	13649						119.62		
	443	49	DARK									
115	12801	11436	12801	11436	12119	8%	14344	17%	154.01	174.82	134.13	21%
	14104	13329	14104	13329	13717	4%			137.01	146.82		
	15970	18424	15970	18424	17197	10%			116.32	93.91		
89.7	16775	17104	16775	17104	16940	1%	16182	7%	108.45	105.39	114.19	10%
	10283	12498										
	16180	14670	16180	14670	15425	7%			114.22	130.33		
109	21312	22195	21312	22195	21754	3%	18905	19%	72.45	66.69	90.02	37%
	15506	13282	15506	13282	14394	11%			121.13	147.45		
	21412	19724	21412	19724	20568	6%			71.78	83.70		
99.7	13861	16815	13861	16815	15338	14%	17181	18%	140.00	108.08	104.69	28%
	13515	18272	13515	18272	15894	21%			144.39	95.18		
	20221	20404	20221	20404	20313	1%			80.05	78.73		
97	18686	17207	18686	17207	17947	6%	16782	10%	91.78	104.45	108.39	15%
	1	4	DARK	DARK								
	14549	16684	14549	16684	15617	10%			131.73	109.32		
114	13605	14626	13605	14626	14116	5%	16192	19%	143.23	130.84	114.09	24%
	21319	18334	21319	18334	19827	11%			72.40	94.66		
	14730	14539	14730	14539	14635	1%			129.64	131.84		
114	11303	13063	11303	13063	12183	10%	14080	18%	177.04	150.39	137.30	23%
	15898	16683	15898	16683	16291	3%			117.05	109.33		
	11206	16329	11206	16329	13768	26%			178.69	112.74		

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94.7	18926	21163	18926	21163	20045	8%	19194	7%	89.85	73.46	87.75	11%
	18181	18507	18181	18507	18344	1%			95.94	93.23		
	24007	26095										
110	15843	15532	15843	15532	15688	1%	19159	17%	117.62	120.85	88.02	30%
	20946	20502	20946	20502	20724	2%			74.94	78.04		
	22974	24238	22974						61.87			
99.9	14922	17845	23588	17845	20717	20%	18656	15%	58.20	98.80	92.02	23%
	15951	16452	15951	16452	16202	2%			116.52	111.54		
	18241	19861	18241	19861	19051	6%			96.43	82.68		
103	16230	16386	16230	16386	16308	1%	16105	12%	113.72	112.19	114.97	18%
	16278	12726	16278	12726	14502	17%			113.25	155.06		
	16040	18968	16040	18968	17504	12%			115.62	89.52		
103	13409	16633	13409	16633	15021	15%	15933	11%	145.77	109.80	116.70	16%
	16818	14180	16818	14180	15499	12%			108.05	136.09		
	17009	17549	17009	17549	17279	2%			106.27	101.38		
94.2	20081	20420	20081	20420	20251	1%	17087	16%	81.06	78.62	105.55	23%
	14896	17600	14896	17600	16248	12%			127.77	100.93		
	15269	14254	15269	14254	14762	5%			123.66	135.20		
98	21061	22116	21061	22116	21589	3%	17953	19%	74.15	67.19	97.87	29%
	15579	12728	15579						120.36			
	14875	16136	14875	16136	15506	6%			128.00	114.65		
108	16637	17078	16637	17078	16858	2%	19166	10%	109.76	105.63	87.97	18%
	21198	19538	21198	19538	20368	6%			73.22	85.10		
	21071	19471	21071	19471	20271	6%			74.08	85.61		
85.9	18825	18813	18825	18813	18819	0%	17104	12%	90.66	90.75	105.40	18%
	10061	10900										
	15739	15037	15739	15037	15388	3%			118.69	126.20		

Table 22: Li Heparin Plasma

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration			
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV
103	16083	18468	16083	18468	17276	10%	16544	11%	115.18	93.55	110.66	15%
	15080	9547	15080						125.72			
	32906	31177										
98.4	12801	18887		18887			18925	10%		90.16	89.86	18%
	20044	20440	20044	20440	20242	1%			81.33	78.48		
	19595	15659	19595	15659	17627	16%			84.67	119.52		
106	33	2108	DARK				17900	7%			98.32	12%
	18960	18770	18960	18770	18865	1%			89.58	91.10		
	16044	17827	16044	17827	16936	7%			115.58	98.95		
85.5	17979	13635	17979	13635	15807	19%	19194	20%	97.65	142.85	87.75	37%
	18806	24159	18806						90.81			
	22097	23451	22097	23451	22774	4%			67.31	59.02		
115	27651	26758					15870	13%			117.34	18%

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	17887	17328	17887	17328	17608	2%			98.44	103.36		
	14397	13868	14397	13868	14133	3%			133.50	139.91		
89.7	18344	19424	18344	19424	18884	4%	16326	18%	94.58	85.97	112.78	27%
	13236	14299	13236	14299	13768	5%			148.06	134.66		
	22933	22558										
	19465	24794	19465						85.68			
109	82	92	DARK	DARK			19671	1%			84.10	2%
	19722	19825	19722	19825	19774	0%			83.72	82.95		
	18589	18848	18589	18848	18719	1%			92.56	90.47		
99.7	16329	18363	16329	18363	17346	8%	17560	7%	112.74	94.42	101.29	10%
	16687	16545	16687	16545	16616	1%			109.29	110.65		
	16700	18312	16700	18312	17506	7%			109.16	94.84		
97	23986	24975					17511	4%			101.73	6%
	17462	17568	17462	17568	17515	0%			102.16	101.22		
	13804	13384	13804	13384	13594	2%			140.71	146.10		
114	19946	19247	19946	19247	19597	3%	16547	16%	82.05	87.34	110.62	24%
	16412	16490	16412	16490	16451	0%			111.93	111.18		
	16363	15238	16363	15238	15801	5%			112.41	124.00		
114	13405	11411	13405				14251	11%	145.82		135.24	13%
	12619	13629	12619	13629	13124	5%			156.59	142.93		
	23352	21403		21403						71.84		
94.7	10294	16010		16010			19711	13%		115.92	83.80	25%
	21385	20044	21385	20044	20715	5%			71.96	81.33		
	17217	21198	17217	21198	19208	15%			104.36	73.22		
110	11376	11244	11376	11244	11310	1%	18259	32%	175.82	178.04	123.77	42%
	35609	39110										
	20336	22762	20336	22762	21549	8%			79.22	63.16		
99.9	10360	13483					19969	10%			81.88	18%
	18089	18089	18089	18689	18389	2%			96.71	91.75		
	19940	23000	19940	23000	21470	10%			82.10	61.71		
103	18475	16785	18475	16785	17630	7%	18629	13%	93.49	108.36	92.24	21%
	16544	17030	16544	17030	16787	2%			110.65	106.07		
	20622	18459	20622	18459	19541	8%			77.19	93.63		
103	12924	15362	12924	15362	14143	12%	16338	17%	152.29	122.66	112.65	23%
	15672	14991	15672	14991	15332	3%			119.38	126.71		
	18514	18194	18514	18194	18354	1%			93.17	95.83		
94.2	20099	22340	20099	22340	21220	7%	18208	16%	80.93	65.77	95.71	26%
	14464	15635	14464	15635	15050	6%			132.72	119.77		
	21683	23092	21683	23092	22388	4%			69.99	61.16		
98	18434	19697	18434	19697	19066	5%	19349	14%	93.83	83.91	86.55	24%
	15812	17378	15812	17378	16595	7%			117.94	102.91		
	16817	20667	16817	20667	18742	15%			108.06	76.87		
108	16324	19724	16324	19724	18024	13%	17660	12%	112.79	83.70	100.41	17%
	15636	16789	15636	16789	16213	5%			119.76	108.32		
	15926	16269	15926	16269	16098	2%			116.77	113.33		
85.9	17662	18765	17662	18765	18214	4%	17156	8%	100.39	91.14	104.92	11%

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99.9	15663	19063	15663	19063	17363	14%	18507	11%	119.48	88.77	93.23	20%
	18530	20771	18530	20771	19651	8%			93.04	76.45		
	14529	17460										
103	24481	26952					15831	11%	125.14	138.28	117.75	15%
	15133	14000	15133	14000	14567	5%			114.89	96.81		
	16112	18077	16112	18077	17095	8%			124.01	106.93		
103	15517	16938	15517	16938	16228	6%	16923	17%	124.01	106.93	107.06	27%
	12858	11420	12858						153.21			
	20550	18753	20550	18753	19652	6%			77.70	91.24		
94.2	60754	63755					18045	11%	119.32	115.00	97.09	22%
	15678	16101	15678	16101	15890	2%			81.40	79.02		
	20035	20364	20035	20364	20200	1%			99.21	121.57		
98	17798	15464	17798	15464	16631	10%	17912	14%	110.08	120.67	98.23	22%
	16604	15549	16604	15549	16077	5%			70.95	77.93		
	21537	20517	21537	20517	21027	3%			105.71	97.73		
108	17070	17969	17070	17969	17520	4%	19530	10%	67.77	74.05	85.17	18%
	22025	21075	22025	21075	21550	3%			77.92	93.11		
	20518	18522	20518	18522	19520	7%			115.66			
85.9	16036	22501	16036				16732	18%			108.86	25%
	22554	21124		21124						73.72		
	14067	15702	14067	15702	14885	8%			137.46	119.07		

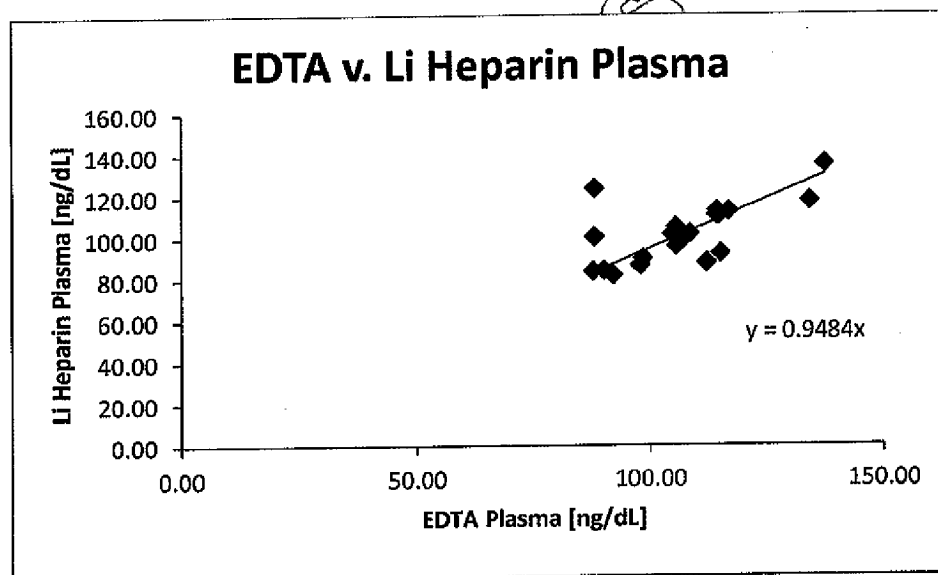
Table 24: EDTA v. Li Heparin v. Serum comparison

Sample ID	Theranos results						
	EDTA CLIA Reported value (ng/dL)	EDTA plasma		Li-Heparin plasma		Serum	
		Theranos results (ng/dL)	% Recovery	Theranos results (ng/dL)	% Recovery	Theranos results (ng/dL)	% Recovery
Patient 1	103	114.6	111%	110.7	107%	88.7	86%
Patient 2 (4P)	98.4	98.5	100%	89.9	91%	104.0	106%
Patient 3	106	106.5	100%	98.3	93%	119.4	113%
Patient 4 (5P)	85.5	112.0	131%	87.8	103%	82.7	97%
Patient 5	115	134.1	117%	117.3	102%	115.5	100%
Patient 6	89.7	114.2	127%	112.8	126%	107.2	119%
Patient 7 (14P)	109	90.0	83%	84.1	77%	95.9	88%
Patient 8	99.7	104.7	105%	101.3	102%	92.6	93%
Patient 9	97	108.4	112%	101.7	105%	107.3	111%
Patient 10	114	114.1	100%	110.6	97%	103.3	91%
Patient 11	114	137.3	120%	135.2	119%	144.0	126%

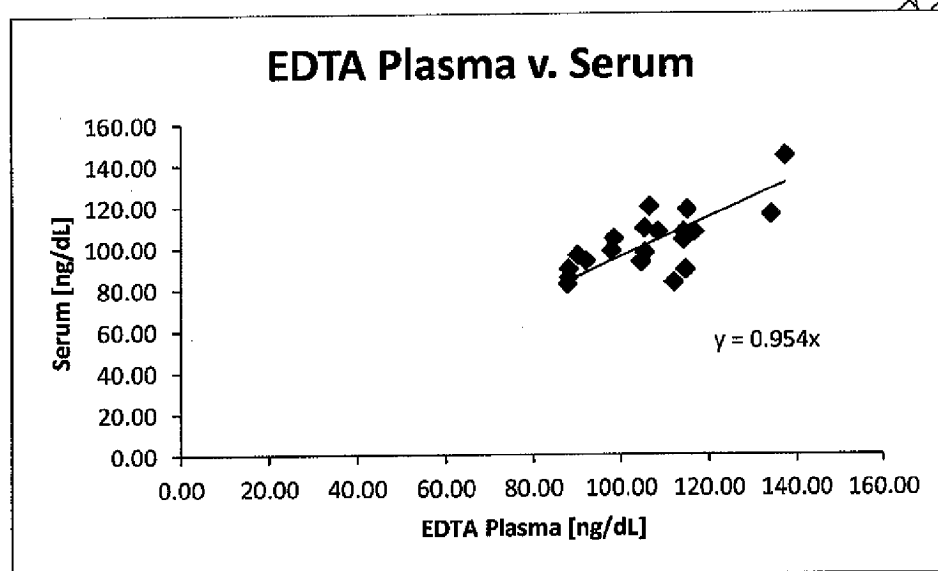
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Patient 12	94.7	87.7	93%	83.8	88%	82.2	87%
Patient 13	110	88.0	80%	123.8	113%	89.3	81%
Patient 14	99.9	92.0	92%	81.9	82%	93.2	93%
Patient 15 (15P)	103	115.0	112%	92.2	90%	117.7	114%
Patient 16	103	116.7	113%	112.7	109%	107.1	104%
Patient 17	94.2	105.6	112%	95.7	102%	97.1	103%
Patient 18	98	97.9	100%	86.5	88%	98.2	100%
Patient 19	108	88.0	81%	100.4	95%	85.2	79%
Patient 20	85.9	105.4	123%	104.9	123%	108.9	127%
Average recovery			106%		100%		101%

Figure 9: EDTA v. Li Heparin Plasma



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Figure 10: EDTA Plasma v Serum

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11 INTERFERENCE

The recovery of analyte spiked into hemolyzed (250mg/dL Hemoglobin), icteric (20mg/dL Bilirubin), and lipemic (501mg/dL Triglycerides) serum samples were evaluated on the Theranos System and compared to predicate method. Hemolyzed samples didn't show significant difference with hemoglobin up to 250mg/dL. Icteric samples didn't show significant difference with Bilirubin up to 20mg/dL. Lipemic samples had slightly low recovery at low T3 level with triglycerides level at 501mg/dL. Results of lipemic samples should be interpreted with cautions.

Table 25: Hemolyzed Samples

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration		Mean	%CV	Reported %Recovery
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2			
<40	35746	45424	35746	45424	40585	17%	36414	13%	OORL	OORL	OORL	OORL	OORL
	36394	36113	36394	36113	36254	1%			OORL	OORL			
	31828	32976	31828	32976	32402	3%			OORL	OORL			
74.2	20009	22730	20009	22730	21370	9%	24093	12%	81.59	63.35	55.32	30%	75%
	26305	27754	26305						43.53				
	24630	26790	24630	26790	25710	6%			52.33	41.12			
127	16209	15160	16209	15160	15685	5%	15868	4%	113.93	124.84	117.37	5%	92%
	15469	15490	15469	15490	15480	0%			121.52	121.29			
	16242	16635	16242	16635	16439	2%			113.60	109.78			
236	6450	6421	6450	6421	6436	0%	6073	10%	302.14	303.32	318.14	9%	135%
	6472	6429	6472	6429	6451	0%			301.26	302.99			
	5547	5120	5547	5120	5334	6%			343.38	366.96			

Table 26: Icteric Samples

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration		Mean	%CV	Reported %Recovery
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2			
<40	29554	31200	29554	31200	30377	4%	37795	19%	OORL	OORL	OORL	OORL	OORL
	33226	41282	33226	41282	37254	15%			OORL	OORL			
	45727	45778	45727	45778	45753	0%			OORL	OORL			
62	17215	18896	17215	18896	18056	7%	21912	21%	104.38	90.09	68.50	44%	110%
	36517	37848											
	25964	25574	25964	25574	25769	1%			45.26	47.27			
170	15251	15992	15251	15992	15622	3%	14941	12%	123.85	116.10	127.26	17%	75%
	16100	16892	16100	16892	16496	3%			115.01	107.36			
	12041	13372	12041	13372	12707	7%			165.16	146.26			
257	7677	6788	7677	6788	7233	9%	7676	15%	259.00	289.02	259.03	13%	101%
	7192	8676	7192	8676	7934	13%			274.65	231.20			
	6384	9340	6384	9340	7862	27%			304.83	215.32			

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Table 27: Lipemic Samples

Reported Value [ng/dL]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration		Mean	%CV	Reported %Recovery
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2			
111	22677	21414	22677	21414	22046	4%	22414	3%	63.68	71.77	65.3	7%	59%
	14514	13376											
	22658	22906	22658	22906	22782	1%			63.80	62.88			
174	13010	12305	13010	12305	12658	4%	12683	18%	151.11	161.07	155.67	19%	89%
	10468	11368	10468	11368	10918	6%			191.99	175.95			
	16266	18331	16266						113.36				
213	5676	7193	5676	7193	6435	17%	8272	22%	336.84	274.62	241.82	23%	114%
	9352	10439	9352	10439	9896	8%			215.05	192.54			
	7192	9778	7192	9778	8485	22%			274.65	205.77			
309	3969	4797	3969	4797	4383	13%	4378	25%	450.96	387.07	416.86	22%	135%
	5608	5506	5608	5506	5557	1%			340.26	345.52			
	3076	3310	3076	3310	3193	5%			552.25	521.04			

12 CROSSREACTIVITY

Cross-reactivity was tested in the assay development. The "Test Substance" was spiked into 3 levels of T3 standards and the % recovery was determined. All standards with spiked cross-reactive substances should recover 100% \pm 25% of analyte. No cross-reactivity was seen with Thyroxine, 3,3',5' Triiodothyronine (Reverse T3), 3-Iodo-L-tyrosine, 3,5-Diiodo-L-thyronine (T2), Sodium Salicylate, PhenylButazone, or Diphenylhydantoin.

Table 28: Cross-reactive Samples

Test Substance	[Test Substance]	[T3]	Signal RLU		Back-Calculated Conc. (ng/dL)		
	ng/dL	ng/dL	Mean RLU	CV%	Mean Conc.	CV%	%Recovery
Control		10.2	892	4%	10.4	4%	102%
		5.2	2465	15%	4.9	9%	94%
		1.7	9997	4%	1.9	4%	112%
		1.2	16967	3%	1.1	4%	92%
		0.6	24548	3%	0.7	4%	117%
Thyroxine (T4)	900	10.2	1093	3%	8.6	2%	84%
		5.2	2209	8%	5.2	5%	100%
		1.2	13854	1%	1.4	1%	117%
3,3',5' Triiodothyronine (Reverse T3)	1.5	10.2	1165	1%	8.2	0%	80%
		5.2	2665	14%	4.7	8%	90%
		1.2	16081	6%	1.2	7%	100%
3-Iodo-L-tyrosine	30	10.2	962	6%	9.7	5%	95%

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3,5-Diiodo-L-thyronine (T2)	30	5.2	2430	3%	4.9	2%	94%
		1.2	13117	5%	1.5	5%	125%
		10.2	1111	6%	8.5	5%	83%
		5.2	3128	0%	4.6	0%	88%
		1.2	13489	4%	1.4	4%	117%
Sodium Salicylate	400000	10.2	1152	10%	8.3	9%	81%
		5.2	2415	5%	4.9	3%	94%
		1.2	14781	3%	1.3	3%	108%
PhenylButazone	300000	10.2	928	16%	10.2	15%	100%
		5.2	1998	2%	5.3	1%	102%
		1.2	10719	9%	1.8	8%	150%
Diphenylhydantoin	300	10.2	1170	5%	8.2	4%	80%
		5.2	2510	8%	4.8	3%	92%
		1.2	14935	9%	1.3	10%	108%

13 SAMPLE STABILITY

The stability of calibrators stored at 4C and room temperature were tested to mimic the conditions that clinical samples may be stored and handled. Samples were transferred from -80C to either room temperature or 4C at the 0hr time point, and stored at those temperatures for the remainder of testing. Overall the analyte stability data indicates that the samples can be processed up to 24 hours when stored at 4 degrees and up to 1 hour when stored at room temperature.

Table 29: Analyte Stability Summary

CLIA reported value (ng/dL)	Theranos results (ng/dL)											
	4C						Room Temperature					
	0hr	1hr	4hr	12hr	24hr	72hr	0hr	1hr	4hr	12hr	24hr	72hr
710	733.25	884.36	925.47	732.56	750.41	845.32	733.25	695.71	784.07	712.66	799.20	770.07
142	120.34	122.14	147.35	131.33	89.98	85.08	120.34	94.10	119.40	159.57	116.32	124.78
71	62.13	83.22	67.54	46.31	73.29	OORL	62.13	50.02	OORL	OORL	48.64	OORL

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Figure 11: Analyte Stability at 4C

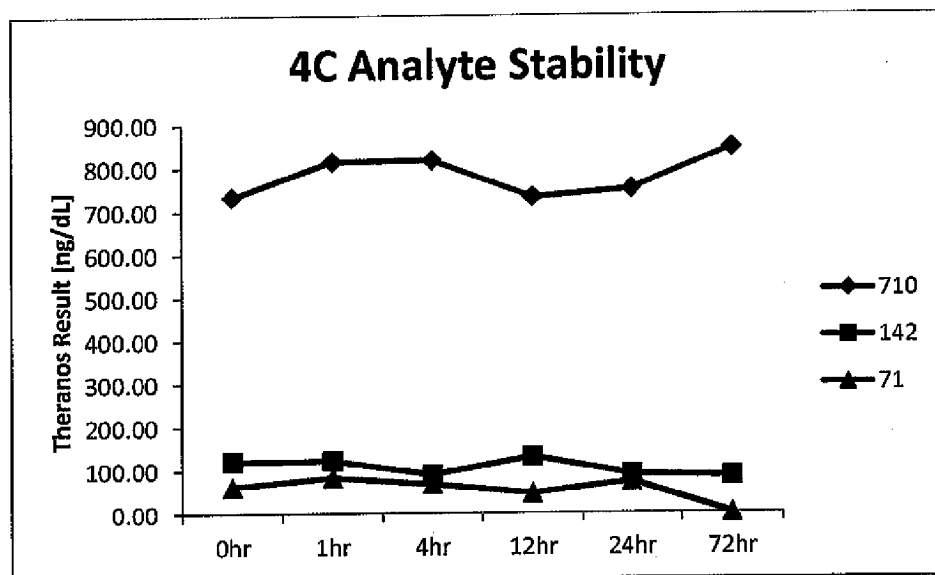
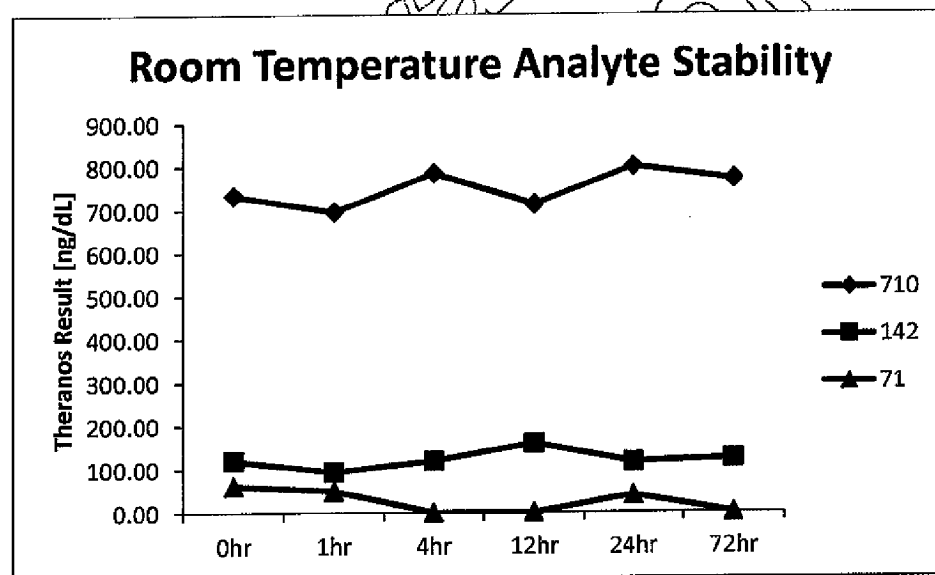


Figure 12: Analyte Stability at Room Temperature

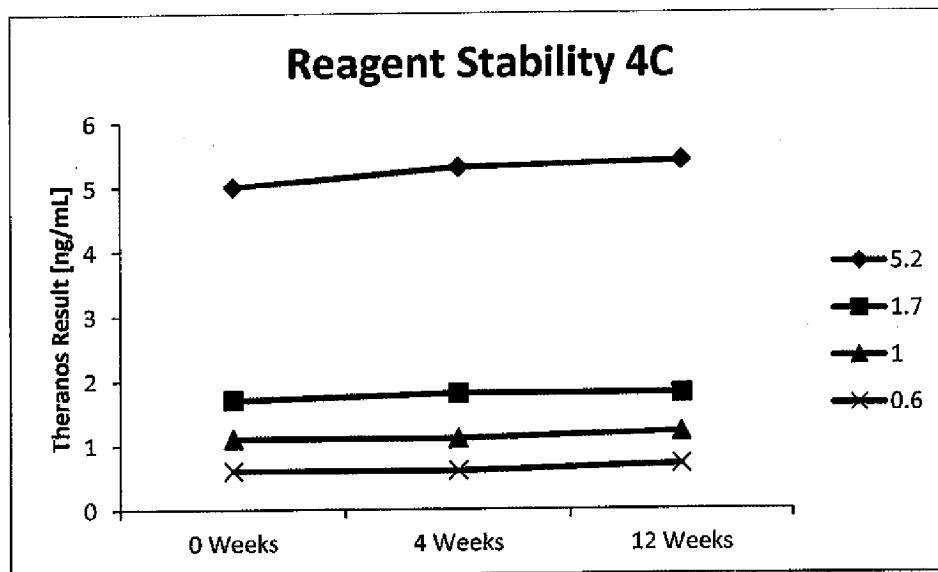


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14 Reagent Stability

Theranos System reagents are stable up to 12 weeks at 4C

Figure 13: Reagent Stability at 4C



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15 REFERENCES

- 15.1 Code of Federal Regulations, Title 42, Chapter IV, Subchapter G, Part 493, Subpart K, Sections 493.1217, 493.1253, and 493.1255.
- 15.2 DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharmaceutical Res.* 2003; 20:1885-1900.
- 15.3 Guidance for Industry: bioanalytical method validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2001.
- 15.4 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.
- 15.5 StatPro (version 1.13.00). Clinical and Laboratory Standards Institute, Wayne, PA. 07/14/2011.
- 15.6 Dexter-Immunoassay (version 1.0). Theranos, Inc., 2009.
- 15.7 EP10-A3, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition, 2006, Clinical and Laboratory Standards Institute, Wayne, PA.
- 15.8 EP15-A2, User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. 2005, Clinical and Laboratory Standards Institute, Wayne, PA.
- 15.9 EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision), 2010, Clinical and Laboratory Standards Institute, Wayne, PA.
- 15.10 EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.
- 15.11 EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003, Clinical and Laboratory Standards Institute, Wayne, PA.
- 15.12 EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.