



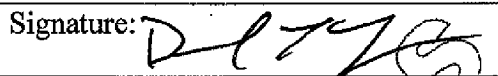
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Author(s):

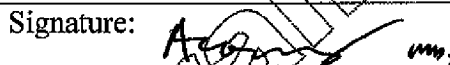
Signature: 	Date: 30 Sep 2013
Name: Michelle Johnson	Title: Immunoassay Research Associate

Reviewer(s)

Signature: 	Date: 9/25/2013
Name: Sharada Sivaraman, PhD	Title: Immunoassay Team Leader

Signature: 	Date: 9/25/2013
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

Signature: 	Date: 9/30/2013
Name: Adam Rosendorff, M.D	Title: Laboratory Director

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 9/19/15

Sunil S. Dhawan M.D.

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1 ASSAY BACKGROUND

Vitamin D is important in overall health and wellness. It is produced naturally when the body is exposed to sunlight, and is also available in supplements and many fortified foods. Vitamin D is converted into a hormone in the body and helps regulate the amounts of calcium in the blood. A deficiency in vitamin D can lead to fatigue, and generalized pain. It is also linked to cancer, asthma, type-II diabetes, high blood pressure, Alzheimer's and autoimmune diseases. Vitamin D toxicity also occurs when too much of the supplement is taken, and can cause high blood calcium. In healthy adults the normal range is between 9.3ng/mL and 47.9ng/mL. Toxicity occurs above 150ng/mL and levels lower than 9.3ng/mL are considered deficient.

1.1 Theranos System Specification

The Theranos Total 25 OH Vitamin D assay determines the total concentration of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 in human plasma or serum. The assay has a reportable range of 10 ng/mL to 150 ng/mL. The ULOQ is 150 ng/ml and LLOQ is 10 ng/ml. The reference range for the assay is 9.3-47.9 ng/ml.

1.1.1 Principle

A biotin-labeled anti-sheep antibody coated on UltraAvidin serves as the capture surface for the competitive ELISA. The sample is diluted and mixed with pepsin in a low pH buffer to remove Vitamin D Binding Protein (VDBP) and other interfering proteins. The mixture is then further diluted into a buffer with pH 8.0 to inactivate the pepsin, and the sheep anti-25-Hydroxyvitamin D3/2 antibody mixture is added and incubated with the sample for 10 minutes. After this incubation, an alkaline phosphatase-labeled 25-Hydroxyvitamin D3 conjugate (25OHD3-AP) is added to the mixture. The reaction mixture is incubated on the capture surface, then the surface is washed and the alkaline phosphatase substrate is incubated on the surface, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

A greater amount of total 25-Hydroxyvitamin D in the sample results in lower binding of the 25OHD3-AP to the capture antibody. Thus the signal generated by the assay is inversely proportional to the concentration of 25-Hydroxyvitamin D in the sample.

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1.2 Reference Assay

Diasorin Liaison was used as a predicate method. The 25 OH vitamin D TOTAL test has a reportable range of 4ng/mL to 150ng/mL and uses only Serum sample type. Hemolyzed (200mg/dL hemoglobin), Lipemic (589 mg/dL triglycerides) and Icteric samples (40mg/dL) show no interference.

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 For the purposes of this Validation Plan, calibration will be carried out for each new lot of reagent cartridges.

3.1.1 At each level 3 cartridge replicates will be tested.

3.1.2 A calibration curve should consist of a blank or zero sample (matrix sample processed without internal standard or a buffer without internal standard) and six to eight non-zero samples covering the expected range, including calibration standards at the LLOQ and ULOQ of the range,

3.1.3 Acceptance criteria: For each run, a minimum of 75% of the back-calculated mean values of the total number of calibration standards in the calibration range should be within $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ and ULOQ standards) of their nominal values, and a minimum of six unique standard concentrations must be within the assay range.

Calibrators were made in depleted serum with Cerilliant certified reference material. Calibrators may be adjusted to the Diasorin instrument as a predicate device. A total of 8 calibrator levels were used to cover the reportable range from 10 ng/mL to 150 ng/mL. Expected LLOQ is 10 ng/mL and expected ULOQ is 150 ng/mL.

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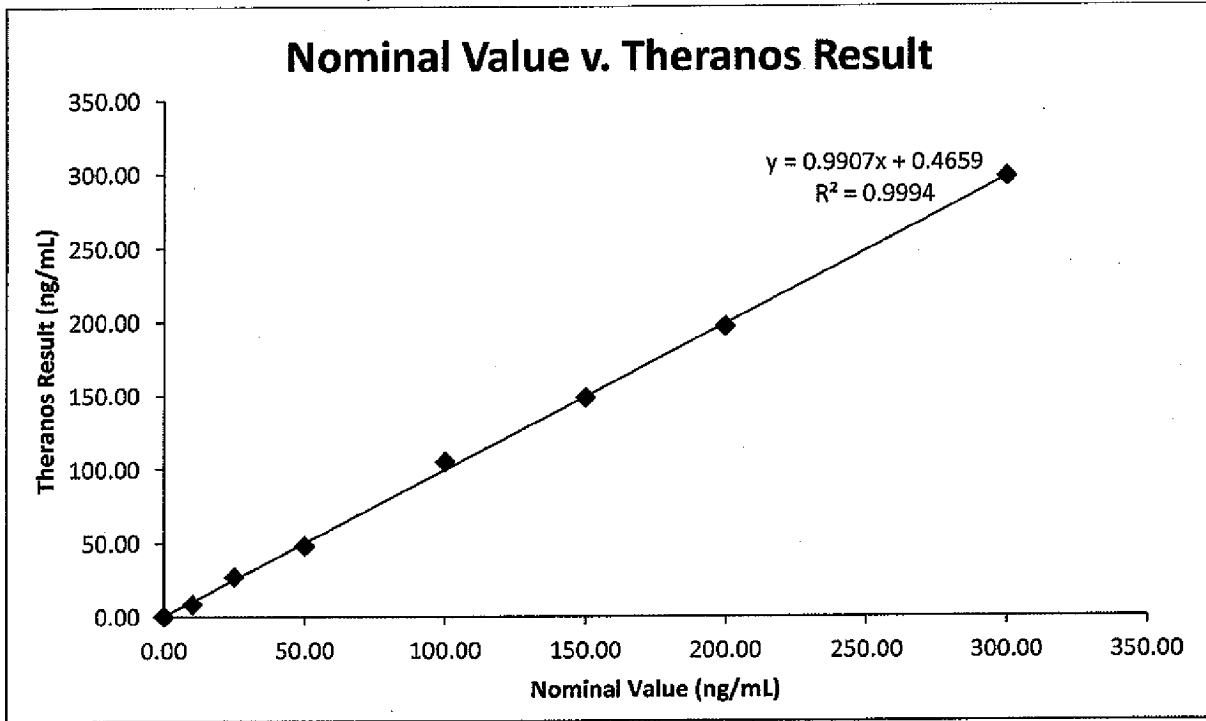
Table 1: Calibration 1 Data (Edison 3.0)

This calibration was used to analyze the Reference Range (3), Accuracy/Comparability (4), Blood Collection Device Comparison (6), Anticoagulant Comparison (7), Interference (9) and Analyte Stability (12). Any tip that resulted in less than 150 RLU is considered a "DARK" tip. These and other obvious outliers, highlighted in red, were excluded from the overall mean and recovery calculations. The %CVs and recovery of each level are well within acceptable ranges.

Nominal Value [ng/mL]	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Dexter Calculated Result				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Concentration		Mean	%CV	%Recovery
									Tip 1	Tip 2			
300.00	4084	3917	4084	3917	4001	3%	3774	15%	281.51	290.02	297.83	8%	99%
	4321	4158	4321	4158	4240	3%			270.33	277.90			
	3225	2940	3225	2940	3083	7%			332.95	OORH			
200.00	6924	7532	6924	7532	7228	6%	6643	22%	190.40	178.32	196.55	19%	98%
	4714	4902	4714	4902	4808	3%			253.82	246.66			
	7738	8049	7738	8049	7894	3%			174.57	169.19			
150.00	11132	9994	11132	9994	10563	8%	9455	17%	129.24	141.73	148.45	16%	99%
	9443	7251	9443	7251	8347	19%			148.61	183.70			
	32688	31218											
100.00	15470	13345	15470	13345	14408	10%	14019	8%	95.42	109.85	104.91	7%	105%
	14402	14689	14402	14689	14546	1%			102.26	100.35			
	12399	13809	12399	13809	13104	8%			117.48	106.41			
50.00	28621	28731	28621	28731	28676	0%	28086	8%	46.64	46.38	47.89	11%	96%
	25476	25521	25476	25521	25498	0%			54.61	54.49			
	30671	29495	30671	29495	30083	3%			42.15	44.67			
25.00	39777	45075	39777	45075	42426	9%	39750	11%	26.92	20.49	26.96	21%	108%
	34863	44437	34863	44437	39650	17%			34.33	21.19			
	36606	37741	36606	37741	37173	2%			31.52	29.80			
10.00	57615	58806	57615	58806	58210	1%	59428	15%	9.56	8.76	8.35	51%	84%
	74761	57356	74761	57356	66058	19%			OORL	9.74			
	47640	60390	47640	60390	54015	17%			17.82	OORL			
0.00	59073	70036	59073	70036	64555	12%	62474	8%	8.58	OORL	OORL	OORL	OORL
	64306	56501	64306	56501	60404	9%			OORL	10.34			
	65526	59401	65526	59401	62464	7%			OORL	8.37			

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Figure 1: Calibration 1



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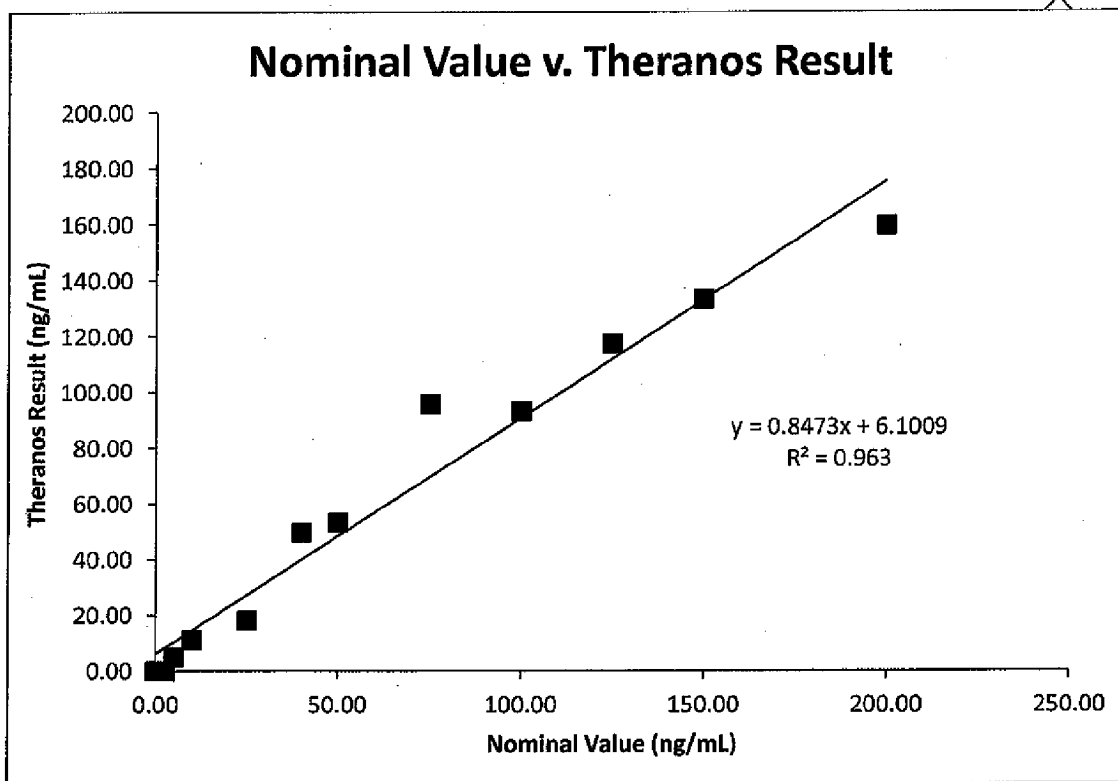
Table 2: Calibration 2 Data (Edison 3.0) From 16 May 2012

This calibration was used to analyze the Dilution Linearity (5) and Cross Reactivity (10). No points were excluded from this data set.

Nominal Value [ng/mL]	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Dexter Calculated Result				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Concentration				%Recovery
									Tip 1	Tip 2	Mean	%CV	
200.00	1807	1665	1807	1665	1736	6%	1692	7%	147.75	162.73	159.63	4%	80%
	1745	1792	1745	1792	1769	2%			153.90	149.19			
	1673	1470	1673	1470	1572	9%			161.80	OORL			
150.00	1790	1749	1790	1749	1770	2%	1978	9%	149.38	153.49	133.28	10%	89%
	2007	2016	2007	2016	2012	0%			131.13	130.47			
	2156	2150	2156	2150	2153	0%			121.16	121.53			
125.00	2234	2259	2234	2259	2247	1%	2220	2%	116.57	115.18	117.38	2%	94%
	2179	2178	2179	2178	2179	0%			119.77	119.83			
	2240	2229	2240	2229	2235	0%			116.24	116.86			
100.00	2533	2618	2533	2618	2576	2%	2763	11%	101.98	98.51	93.13	11%	93%
	3263	3040	3263	3040	3152	3%			78.57	84.43			
	2513	2613	2513	2613	2563	3%			102.83	98.71			
75.00	2581	2651	2581	2651	2616	2%	2691	4%	99.99	97.23	95.72	4%	128%
	2582	2694	2582	2694	2638	3%			99.95	95.62			
	2878	2761	2878	2761	2820	3%			89.31	93.21			
50.00	4722	4783	4722	4783	4753	1%	4803	3%	54.13	53.43	53.21	3%	106%
	4957	4821	4957	4821	4889	2%			51.53	53.00			
	4593	4939	4593	4939	4766	5%			55.66	51.72			
40.00	4935	4982	4935	4982	4959	1%	5139	9%	51.76	51.26	49.68	9%	124%
	4721	4732	4721	4732	4727	0%			54.14	54.01			
	5708	5754	5708	5754	5731	1%			44.61	44.24			
25.00	12684	13202	12684	13202	12943	3%	12354	5%	17.55	16.56	18.21	7%	73%
	11762	12738	11762	12738	12250	6%			19.48	17.44			
	12003	11734	12003	11734	11869	2%			18.95	19.54			
10.00	16867	17896	16867	17896	17382	4%	16780	10%	10.97	9.71	11.08	20%	111%
	18026	18452	18026	18452	18239	2%			9.56	9.07			
	14576	14865	14576	14865	14721	1%			14.21	13.76			
5.00	22725	23160	22725	23160	22943	1%	22649	2%	4.82	4.43	4.89	9%	98%
	22870	22926	22870	22926	22898	0%			4.69	4.64			
	21873	22341	21873	22341	22107	1%			5.60	5.17			
2.50	29153	28210	29153	28210	28682	2%	25334	11%	OORL	OORL	OORL	OORL	OORL
	24283	24081	24283	24081	24182	1%			OORL	OORL			
	22239	24040	22239	24040	23140	6%			5.26	OORL			
0.00	27806	30659	27806	30659	29233	7%	27972	7%	OORL	OORL	OORL	OORL	OORL
	28840	27916	28840	27916	28378	2%			OORL	OORL			
	24753	27858	24753	27858	26306	8%			OORL	OORL			

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Figure 2: Calibration 2



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Table 3: Calibration 3 Data (Edison 3.0) from 26 March 2013

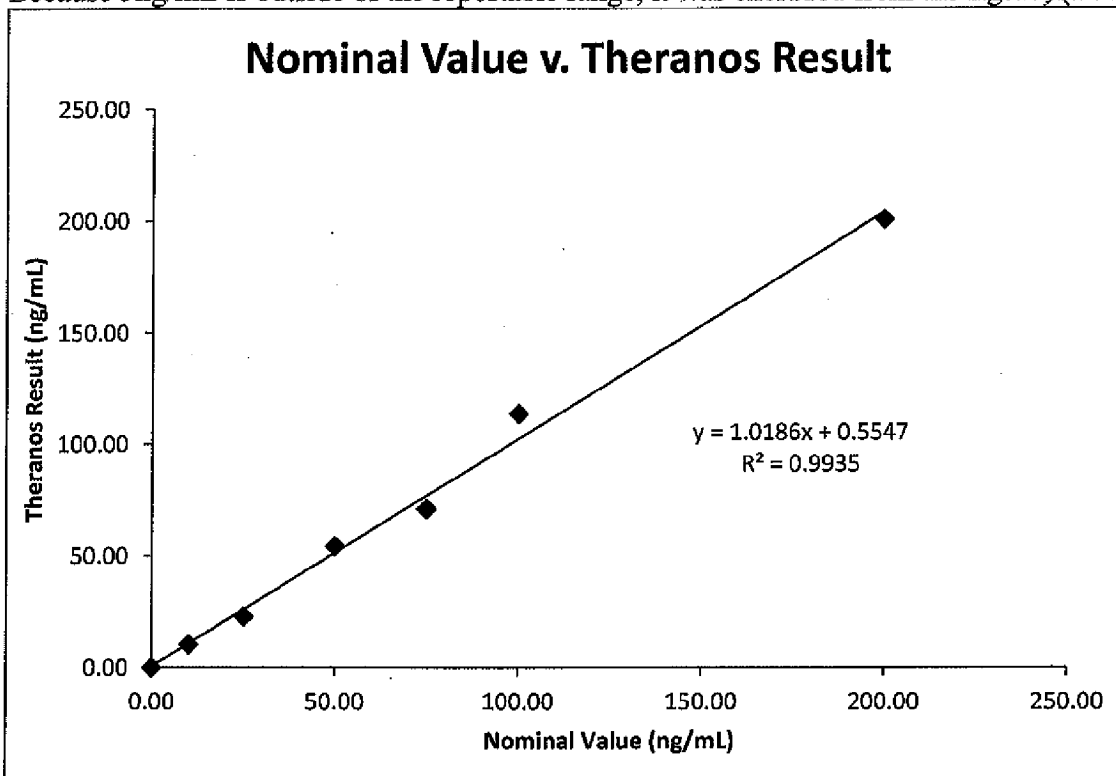
This calibration was used to analyze Accuracy/Comparability (3). Outliers and “DARK” tips were excluded from the mean calculations and overall recoveries. The %CVs and recovery of each level are well within acceptable ranges.

Nominal Value [ng/mL]	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Dexter Calculated Result				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Concentration			%CV	%Recovery
									Tip 1	Tip 2	Mean		
200.00	11433	11383	11433	11383	11408	0%	10349	10%	167.55	168.77	200.68	8%	100%
	8775	9491	8775	9491	9133	6%			OORL	OORH			
	10492	10520	10492	10520	10506	0%			195.31	194.31			
100.00	2495	10225		10225	6360	86%	15187	13%		205.66	113.61	46%	114%
	17898	17658	17898	17658	17778	1%			94.84	96.18			
	14967	5	14967	DARK	7486	141%			115.58				
75.00	22892	9	22892	DARK	11451	141%	24201	10%	74.59		70.88	9%	95%
	21181	24000	21181	24000	22591	6%			80.23	71.42			
	25308	27623	25308	27623	26466	6%			68.08	63.01			
50.00	30542	32419	30542	32419	31481	4%	32840	10%	57.76	54.90	54.30	9%	109%
	32488	38016	32488	38016	35252	11%			54.80	48.01			
	28544	35029	28544	35029	31787	14%			61.24	51.42			
25.00	42019	89803		89803	65911	51%	89780	5%		22.69	22.69	5%	91%
	96279	87964	96279	87964	92122	6%			21.17	23.15			
	48610	85074		85074	66842	39%				23.90			
10.00	153204	164256	153204	164256	158730	5%	167914	6%	11.99	10.68	10.26	11%	103%
	180357	178415	180357	178415	179386	1%			8.89	9.10			
	165812	165437	165812	165437	165625	0%			10.50	10.54			
5.00	199361	208864	199361	208864	204113	3%	207285	13%	OORL	OORL	OORL	OORL	OORL
	174656	183160	174656	183160	178908	3%			9.51	8.59			
	230226	247440	230226	247440	238833	5%			OORL	OORL			
0.00	248585	262475	248585	262475	255530	4%	257229	8%	OORL	OORL	OORL	OORL	OORL
	234057	235607	234057	235607	234832	0%			OORL	OORL			
	281731	280916	281731	280916	281324	0%			OORL	OORL			

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Figure 3: Calibration 3

Because 5ng/mL is outside of the reportable range, it was excluded from the figure below.



Final


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Table 4: Calibration 4 Data (Edison 3.5) For Precision Analysis

This calibration was used to qualify the Theranos Edison v.3.5 System. Outliers and “DARK” tips were excluded from the mean calculations and overall recoveries. The %CVs and recovery of each level are well within acceptable ranges.

Nominal Value [ng/mL]	All Points		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Dexter Calculated Result				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Concentration			%CV	%Recovery
									Tip 1	Tip 2	Mean		
300.00	7625	8116	7625	8116	7871	4%	7230	9%	260.95	236.45	285.54	15%	95%
	7536	6620	7536	6620	7078	9%			266.08	336.73			
	6586	6895	6586	6895	6741	3%			340.24	311.15			
200.00	6962	6877					9203	10%	178.99	172.27	197.20	16%	99%
	9895	10192	9895	10192	10044	2%			213.43	240.44			
	8698	8028	8698	8028	8363	6%							
150.00	11447	11334	11447	11334	11391	1%	11263	15%	149.07	150.88	152.05	21%	101%
	12671	13372	12671	13372	13022	4%			132.11	124.10			
	8781	9972	8781	9972	9377	9%			210.56	177.19			
100.00	14801	17035	14801	17035	15918	10%	15016	21%	110.54	94.41	108.76	31%	109%
	19514	9891	19514	9891	14703	46%			81.11	179.09			
	14155	14697	14155	14697	14426	3%			116.27	111.42			
50.00	31239	34009	31239	34009	32624	6%	30433	17%	46.31	41.28	47.91	25%	96%
	35728	33725	35728	33725	34727	4%			38.48	41.76			
	23824	24074	23824	24074	23949	1%			64.61	63.83			
25.00	38009	38293	38009	38293	38151	1%	44824	19%	35.07	34.67	26.47	35%	106%
	54317	53520	54317	53520	53919	1%			16.89	17.62			
	39982	66	39982	DARK	39982				32.37				
10.00	52537	61841	52537	61841	57689	10%	62044	10%	17.61	10.16	9.98	34%	100%
	58834	65536	58834	65536	62185	8%			12.82	OORL			
	61293	71221	61293	71221	66257	11%			10.65	OORL			
0.00	73543	70421	73543	70421	71982	3%	77742	19%	OORL	OORL	OORL	OORL	OORL
	64212	73795	64212	73795	69004	10%			8.02	OORL			
	77136	107343	77136	107343	92240	23%			OORL	OORL			

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Figure 4: Calibration 4

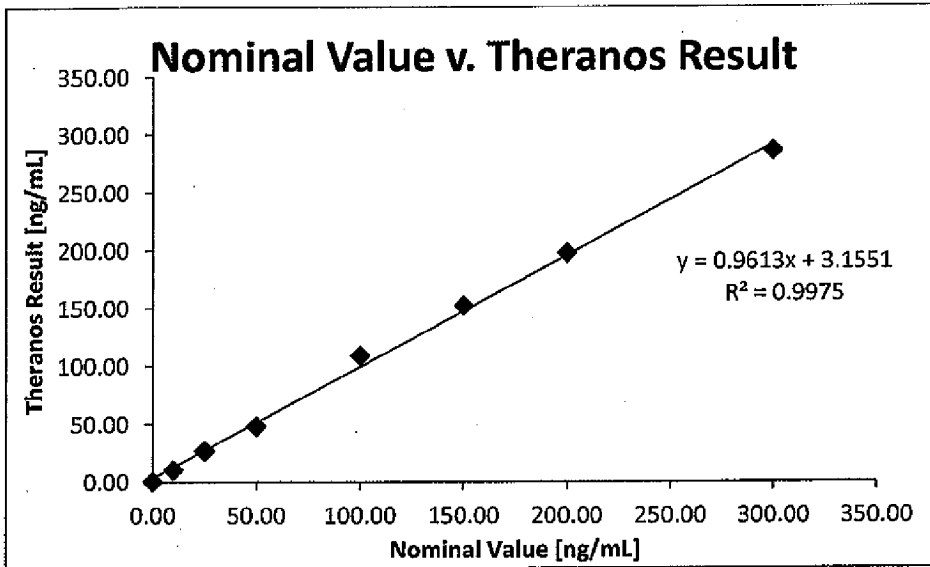
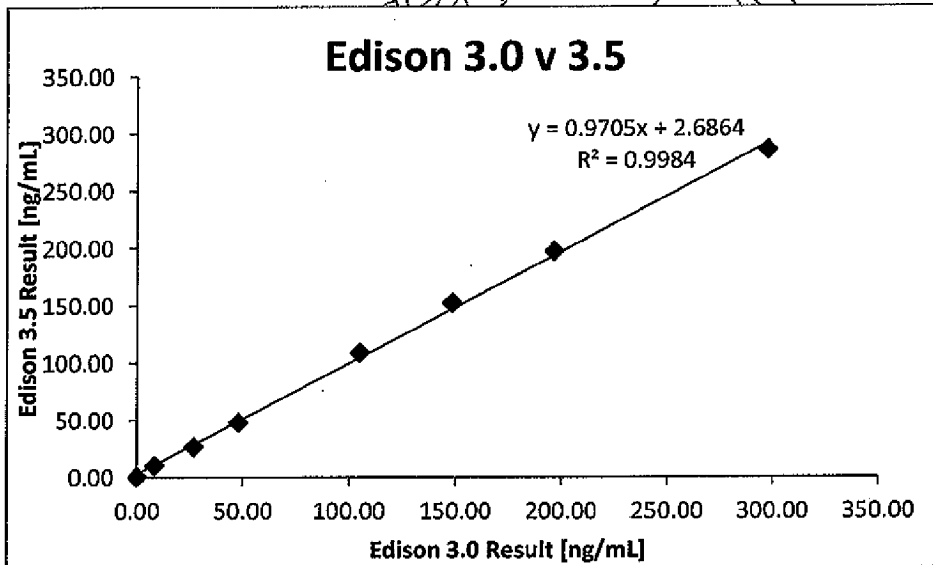



Figure 5: Edison v.3.5 qualification against Edison v.3.0




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4 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 manufacturer’s claims. 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. Review of literature suggested that the normal range of 25OH Vitamin D in blood is between 30ng/mL and 74ng/mL. The reference range measured by the predicate method, Diasorin ranges from 9.3-47.9 ng/mL. Of the 20 normal patient samples, 3 were above the reference range. All but one sample was confirmed on the predicate method.

Table 5: Normal Patient Sample

Sample ID	Reported Value (ng/mL)	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Dexter Calculated Result				
		Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
Patient 1	58.5	21414	22698	21414	22698	22056	4%	22932	5%	67.55	63.07	62.29	6%	106%
		22157	22514	22157	22514	22336	1%			64.90	63.68			
		24009	24800	24009	24800	24405	2%			58.88	56.53			
Patient 2	29.3	39024	40720	39024	40720	39872	3%	37834	9%	27.96	25.67	29.66	19%	101%
		39435	41150	39435	41150	40293	3%			27.39	25.12			
		32823	33851	32823	33851	33337	2%			37.94	36.08			
Patient 3	32.4	27709	26630	27709	26630	27170	3%	26443	17%	48.80	51.51	52.00	24%	160%
		31708	30269	31708	30269	30989	3%			40.06	42.99			
		21324	21016	21324	21016	21170	1%			67.88	69.02			
Patient 4	28	43900	46397	43900	46397	45149	4%	40625	13%	21.80	19.08	25.80	28%	92%
		43318	41652	43318	41652	42485	3%			22.47	24.49			
		34979	33504	34979	33504	34242	3%			34.14	36.69			
Patient 5	23.8	34968	33206	34968	33206	34087	4%	33440	24%	34.16	37.23	36.81	43%	155%
		25385	22904	25385	22904	24145	7%			54.86	62.38			
		42164	42011	42164	42011	42088	0%			23.85	24.04			
Patient 6	39.8	34231	35328	34231	35328	34780	2%	36806	11%	35.41	33.56	31.21	20%	78%
		31664	37176	31664	37176	34420	11%			40.15	30.64			
		42997	39437	42997	39437	41217	6%			22.85	27.39			
Patient 7	31.6	26444	6	26444	DARK	26444		29421	12%	52.00		44.83	17%	142%
		26225	28182	26225	28182	27204	5%			52.57	47.66			
		33321	32934	33321	32934	33128	1%			37.02	37.73			
Patient	57.4	23282	23287	23282	23287	23285	0%	19460	16%	61.16	61.14	75.23	16%	131%

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		18034	18078	18034	18078	18056	0%			81.65	81.44			
		16221	17858	16221	17858	17040	7%			91.04	82.50			
Patient 9	27	39804	45960	39804	45960	42882	10%	40398	16%	26.89	19.54	26.10	35%	97%
		47026	43180	47026	43180	45103	6%			18.44	22.63			
		35697	30718	35697	30718	33208	11%			32.96	42.06			
Patient 10	33.9	39509	38531	39509	38531	39020	2%	36362	8%	27.29	28.65	31.90	15%	94%
		34371	33181	34371	33181	33776	2%			35.17	37.28			
		33550	39031	33550	39031	36291	11%			36.61	27.95			
Patient 11	33.8	50603	55949	50603	55949	53276	7%	46653	14%	15.05	10.74	18.82	35%	56%
		42049	44821	42049	44821	43435	5%			23.99	20.77			
		39845	3	39845	DARK	39845				26.83				
Patient 12	28.9	43036	42471	43036	42471	42754	1%	39427	7%	22.80	23.48	27.40	15%	95%
		36888	36531	36888	36531	36710	1%			31.08	31.63			
		37148	40486	37148	40486	38817	6%			30.69	25.98			
Patient 13	40.1	23947	28410	23947	28410	26179	12%	33614	23%	59.07	47.13	36.50	39%	91%
		33556	30056	33556	30056	31806	8%			36.60	43.45			
		41184	44528	41184	44528	42856	6%			25.08	21.09			
Patient 14	27.9	39268	40708	39268	40708	39988	3%	40064	8%	27.62	25.69	26.54	16%	95%
		43937	43318	43937	43318	43628	1%			21.76	22.47			
		36479	36674	36479	36674	36577	0%			31.71	31.41			
Patient 15	42.5	32604	36920	32604	36920	34762	9%	28254	22%	OORH	57.34	47.50	29%	112%
		4080	24523		24523	24523								
		24329	22893	24329	22893	23611	4%			57.92	62.42			
Patient 16	52	41310	37957	41310	37957	39634	6%	43800	13%	24.92	29.48	21.91	28%	42%
		10341	51642		51642	51642				OORH	14.15			
		47229	40863	47229	40863	44046	10%			18.23	25.49			
Patient 17	28.2	36483	33557	36483	33557	36020	2%	32155	14%	31.71	33.18	39.20	26%	139%
		23867	30159	23867	30159	27013	16%			59.32	43.23			
		33495	33371	33495	33371	33433	0%			36.71	36.93			
Patient 18	46.3	23325	34519	23325	34519	28922	27%	32807	15%	61.02	34.92	37.97	29%	82%
		34749	35731	34749	35731	35240	2%			34.53	32.90			
		32655	35863	32655	35863	34259	7%			38.25	32.69			
Patient 19	23.3	27961	33598	27961	33598	30780	13%	33696	12%	48.19	36.53	36.35	22%	156%
		4	61	DARK	DARK									
		37067	36157	37067	36157	36612	2%			30.81	32.22			
Patient 20	25.6	35808	36627	35808	36627	36218	2%	34822	8%	32.78	31.48	34.40	15%	134%
		32543	30322	32543	30322	31433	5%			38.46	42.88			
		35461	38170	35461	38170	36816	5%			33.34	29.17			

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5 PRECISION

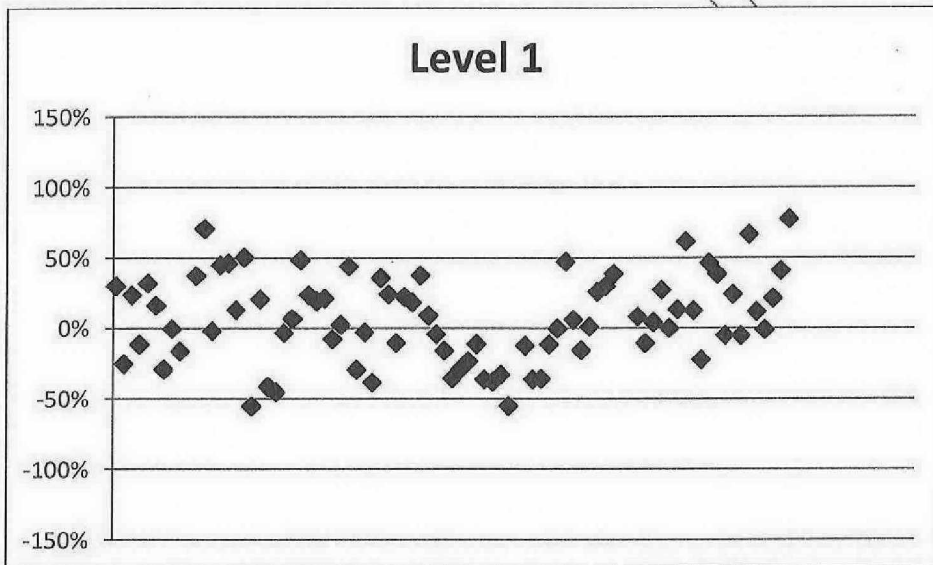
The precision of this assay was tested over 5 days in a morning and evening shift. One run consisted of 9 total replicates at each of 3 analyte levels. In each shift 2 runs were performed. The data shows the precision of the assay on the Theranos System.

Table 6: Precision Summary

Level (ng/ml)	Precision%
15	20.5
33.5	21.3
76.3	9.5

Internal

Figure 6: Level 1 Precision % from Mean



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Figure 7: Level 2 Precision % from Mean

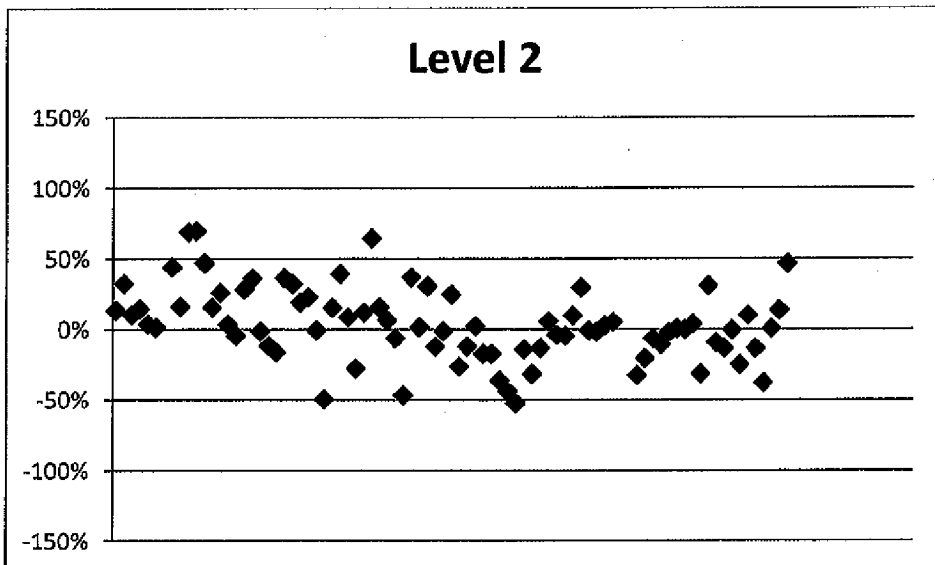
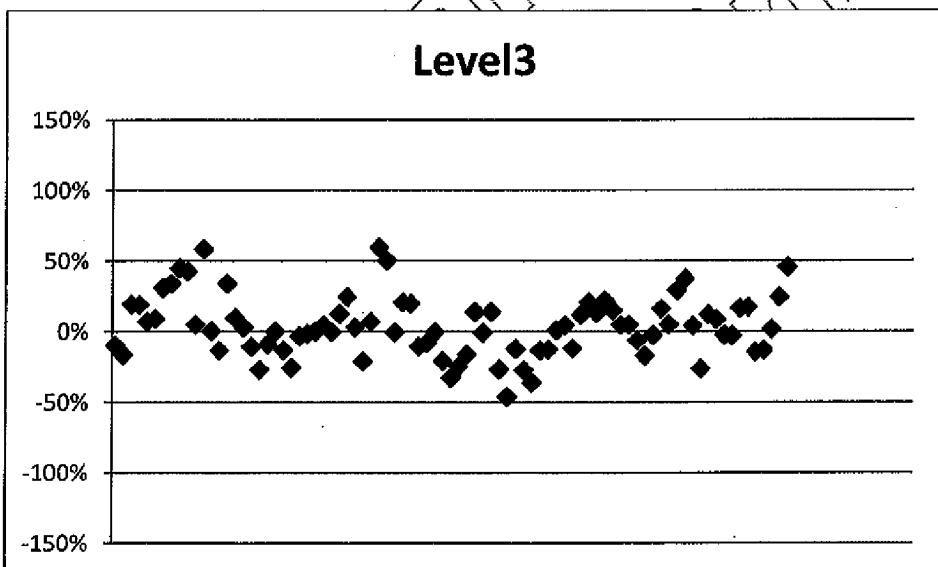


Figure 8: Level 3 Precision % from Mean



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6 ACCURACY/COMPARABILITY 1 (Edison 3.0)

To test the accuracy of the assay on the Theranos System, 111 unique patient samples were screened on the predicate method (Diasorin Liaison) and testing on the Theranos System. Of these samples 42 were within the reference range (30-74 ng/ml), 30 were below the reference range, and 39 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. StatPro 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.

Table 7: Clinical Correlation (Historical)

Historical data from the Theranos Assay Development Report was used.

Sample ID	Reported Value [ng/mL]	Theranos Result [ng/mL]	% Recovery
01	14.4	29.25	203%
02	9.43	16.77	178%
06	17.2	18.11	105%
10	25.4	29.48	116%
11	36	36.91	103%
16	24.4	35.65	146%
20	28.4	22.13	78%
22	41.9	41.76	100%
25	29.2	36.35	124%
27	34.3	41.30	120%
30	27.5	49.18	179%
31	44.3	56.65	128%
33	46.1	51.43	112%
36	59.1	OORL	NA
41	56.5	71.18	126%
87	20.9	20.98	100%
91	33.6	77.24	230%
94	60.3	71.39	118%
95	56.3	87.90	156%
97	54.7	73.93	135%

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102	44.6	68.55	154%
104	63.9	54.38	85%
105	120	124.92	104%
210	82.5	87.74	106%
212	79.9	66.07	83%
214	75.4	77.92	103%
215	71.8	82.98	116%
217	65.6	73.22	112%
218	69.4	69.07	100%
219	60.7	61.11	101%

Table 8: Clinical Correlation

Sample ID	Reported Value [ng/mL]	Theranos Result [ng/mL]	% Recovery
32	51.9	16.24	31%
35	47.8	11.11	23%
37	61.1	22.88	37%
73	47.9	20.77	43%
74	51.4	40.72	79%
75	39.8	26.11	66%
76	55	72.96	133%
79	57.4	53.25	93%
80	66.3	58.73	89%
81	35.6	22.89	64%
84	35.9	21.81	59%
98	42.7	42.79	100%
99	46.6	35.82	77%
100	33.7	31.05	92%
101	33.6	31.17	93%
103	46.4	29.72	64%
216	67.8	75.00	111%
8/31/2013 - 2	36.1	27.73	77%
8/31/2013 - 8	30.1	35.67	119%
8/31/2013 - 10	31.4	28.64	91%
8/31/2013 - 12	38.9	27.36	70%
8/31/2013 - 16	33.8	32.19	95%
3	10.2	OORL	NA
8	14.5	OORL	NA
9	13.5	19.01	141%
13	20	17.69	88%
15	29	12.34	43%
17	22.5	12.72	57%
18	26.5	23.22	88%
28	29	25.46	88%
29	29.8	28.25	95%
89	20.9	OORL	NA
In-House 8	11.9	14.80	124%
In-House 12	17.5	14.72	84%

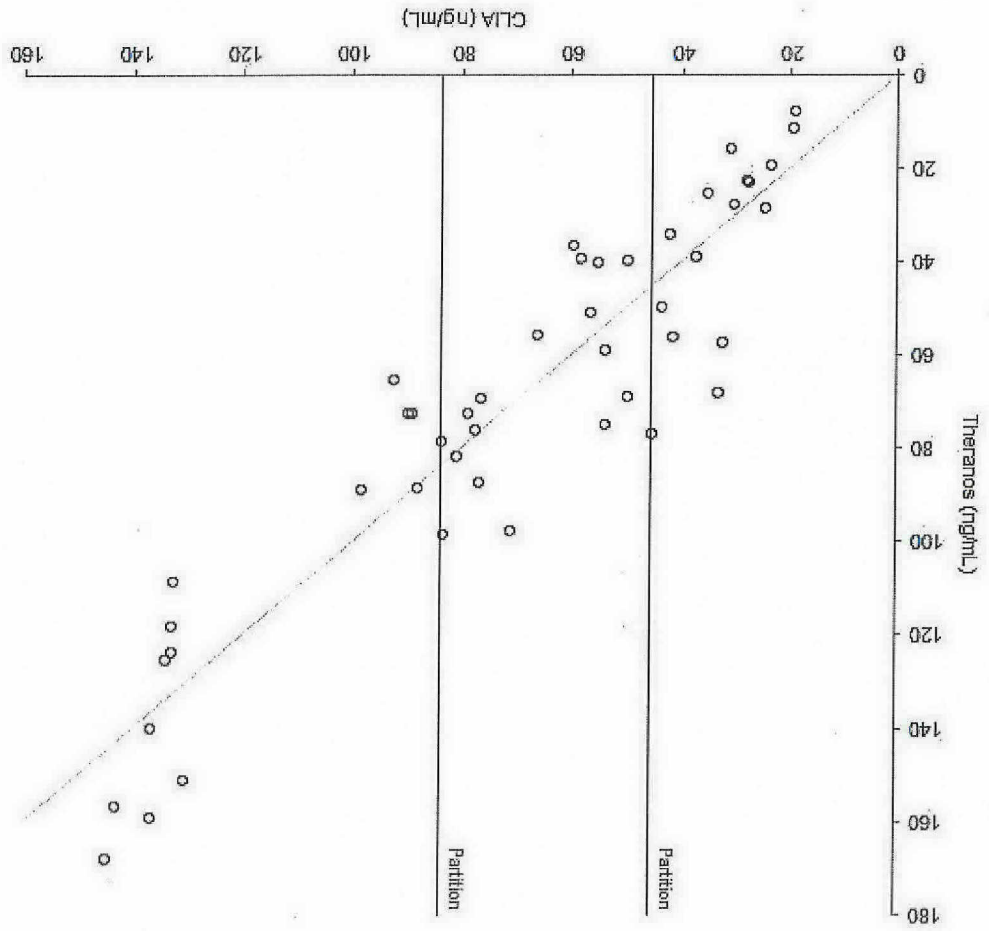
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In-House 13	18	26.59	148%
In-House 14	13.7	19.12	140%
In-House 17	24.9	31.02	125%
In-House 18	9.6	OORL	NA
8/31/2013 - 1	19.4	OORL	NA
8/31/2013 - 3	28.1	44.84	160%
8/31/2013 - 4	24	28.47	119%
8/31/2013 - 5	22.8	42.35	186%
8/31/2013 - 6	16.8	17.90	107%
H2	79.9	104.57	131%
H6	145	136.38	94%
H8	148	133.89	90%
H9	119	125.51	105%
H10	128	119.49	93%
H11	128	113.43	89%
H12	88.1	92.39	105%
H13	125	138.81	111%
H14	128	141.29	110%
H15	76.4	97.15	127%
H16	106	102.61	97%
H17	100	118.21	118%
H18	87.2	101.15	115%
H19	68.1	83.89	123%
H20	93.5	93.25	100%
H21	89.6	74.52	83%
H22	83.1	75.79	91%
H23	83.6	76.57	92%
H24	80.8	75.59	94%
H25	49.5	43.12	87%
H26	76.3	77.14	101%
H27	77.5	79.62	103%
H28	76.7	79.24	103%
H29	71	57.83	81%
H30	88	77.76	88%
H34	104	92.17	89%
H36	104	32.88	32%
H49	140	133.94	96%
H50	145	157.71	109%
H51	143	164.66	115%
H53	137	131.62	96%
H54	134	155.33	116%
H55	131	143.42	109%
H56	129	149.20	116%
H57	124	124.69	101%
H58	129	159.86	124%
H59	143	184.84	129%
H60	133	137.71	104%

Sample ID	Reported Value [ng/mL]		Data-Used for Method Comparison	
	Predicate Method	Theranos System	Theranos-Corrected	Predicate Method
H10	30.4	38.1	27.9	19.1
H16	24.7	39.0	28.6	19.6
H20	23.7	27.5	19.3	23.7
H22	42.2	45.7	34.1	24.7
H25	27.7	31.8	22.8	27.7
H30	27.9	31.4	22.4	27.9
H31	43.4	64.6	49.6	30.4
H36	58.1	52.1	39.3	31.0
H41	53.3	53.2	40.2	38.7
H70	19.1	13.5	7.8	33.2
H91	31.0	23.2	15.7	35.1
H94	59.7	48.5	36.4	37.2
H95	53.8	75.9	58.8	41.5
H70	53.9	95.5	74.9	42.2
H90	56.4	66.3	51.0	43.4
H80	66.1	72.0	52.6	45.3
H10	35.1	35.0	28.3	49.5
H40	37.2	51.6	38.8	49.6
H90	19.6	17.9	11.4	53.6
H90	41.5	72.6	56.1	53.9
H90	45.3	97.9	76.8	55.3
H00	33.2	87.2	68.0	58.4
H01	32.7	74.0	57.3	38.1
H03	49.6	88.1	68.7	59.7
H02	78.8	92.7	72.5	66.1
H08	143.0	195.8	156.8	71.0
H17	98.4	112.8	89.0	76.3
H18	89.0	92.5	72.4	76.7
H20	92.6	84.0	65.4	77.5
H21	89.6	92.6	72.4	78.8
H22	83.1	124.6	98.6	80.8
H23	83.6	100.1	78.6	83.1
H24	80.8	103.8	81.6	83.6
H25	49.5	52.7	39.8	88.0
H26	76.3	88.5	69.1	89.0
H27	77.5	97.0	76.0	89.6
H28	76.7	110.5	87.1	92.6
H29	71.0	123.3	97.6	98.4
H30	88.0	112.2	88.5	130.5
H30	145.0	209.2	167.8	132.5
H33	136.5	198.6	159.2	133.0
H34	136.5	175.4	140.2	133.0
H35	133.0	148.2	117.9	134.0
H36	130.5	188.9	151.2	136.5
H37	134.0	157.3	125.3	136.5
H38	132.5	136.6	108.4	143.0
H60	133.0	155.3	123.7	145.0

Table 9: Bias Correction

25OHVtD Total Report Validation Document		25OHVtD Total ELISA Assay Validation Report on Edison 3.X Theranos System	
		Document Number: Revision: A	Effective Date:



CLSI guideline EP09-A2-IR section 4.2

Scatter plot

Figure 9: Scatter Plot CLSI guideline EP09-A2-IR section 4.2

Method Comparison			
Level (ng/mL)	10	20	30
Precision (%)	20	21	10
Total Error (%)	35	35	25
Allowable Bias (%)	15	14	15

Table 10: Method Comparison

theranos		25OHvID Total Report	
Document Number:	Revision: A	Validation Document	
		Effective Date:	
25OHvID Total ELISA Assay Validation Report on Edison 3.X Theranos System			


	25OHVitD Total Report	Document Number: Revision: A
	Validation Document	Effective Date:
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Figure 10: Difference Plot CLSI guideline EP09-A2-IR section 4.2

Difference plot

CLSI guideline EP09-A2-IR section 4.2

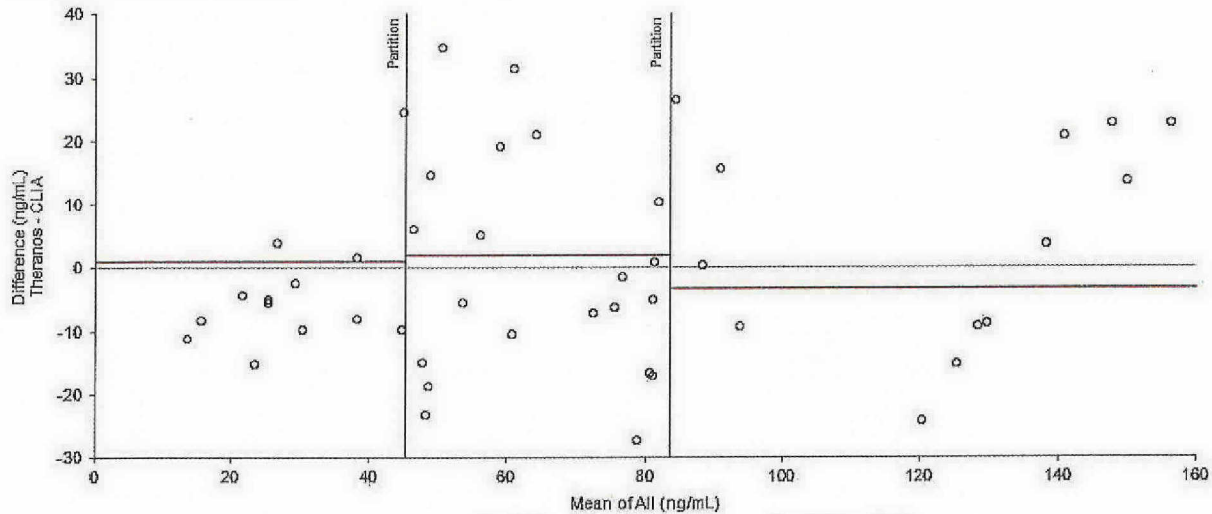


Figure 11: Adequate Range Test EP09-A2-IR section 4.5 and Partitioned Differences CLSI guideline EP09-A2-IR section 6.2

Adequate range test

CLSI guideline EP09-A2-IR section 4.5

$$r = 0.927$$

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

Partitioned differences

CLSI guideline EP09-A2-IR section 6.2

Partition	n	Mean difference	SD
< 45.3	15	1.076296408	13.98798234
≥ 45.3 and < 83.6	17	1.904036590	16.32018060
≥ 83.6	15	-3.22910001	16.68607968


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Figure 12: 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
	10.00000000	1.076296408	3.611681510	569990020 to 8.82258	1.500000000
	30.00000000	1.076296408	3.611681510	569990020 to 8.82258	4.500000000
	100.00000000	-3.229100001	4.308327250	469542944 to 6.0113	15.000000000

Difference is less than allowable bias: 15%.

7 DILUTION LINEARITY

The dilution linearity was tested in replicates of 2. A clinical sample with high levels of 25OH Vitamin D was diluted with a low level sample to create a dilution set. Nominal values were calculated using the reported value of the high patient sample. 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 and ULOQ standards) of their nominal value, and when plotted, the R^2 value should be greater than 0.95.

Calculated concentrations are based on the average RLU of 2 cartridges excluding outliers and dark count tips. When comparing the nominal values to the Theranos results, the linearity is acceptable.

Table 11: Dilution Linearity

Outliers and "DARK" tips and obvious outliers (highlighted in red) were excluded from the mean, %CV and %Recovery calculations.

Sample ID	Reported Value (ng/mL)	Dexter Calculated Result												
		All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Concentration			%CV	%Recovery
		Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean		
High Patient	108.25	2537	2474	2537	2474	2506	2%	2400	6%	101.81	104.54	107.94	6%	100%
		2351	2239	2351	2239	2295	3%			110.35	116.29			
4/7	61.21	3966	3973	3966	3973	3970	0%	3953	1%	64.53	64.41	64.74	1%	106%
		3921	752	3921		3921				65.27	OORH			
1/3	37.69	6886	7125	6886	7125	7006	2%	6344	14%	36.64	35.32	39.96	15%	106%
		5217	6148	5217	6148	5683	12%			48.92	41.30			
1/4	25.93	7350	8144	7350	8144	7747	7%	8246	8%	34.15	30.49	30.07	9%	116%
		8771	8717	8771	8717	8744	0%			28.02	28.22			
1/5	20.05	12037	12055	12037	12055	12046	0%	11410	7%	18.88	18.84	20.29	10%	101%
		10315	11231	10315	11231	10773	6%			23.08	20.71			
1/8	14.17	11746	15972	11746	15972	13859	22%	14788	14%	19.52	12.15	13.88	26%	98%
		15492	15943	15492	15943	15718	2%			12.83	12.19			


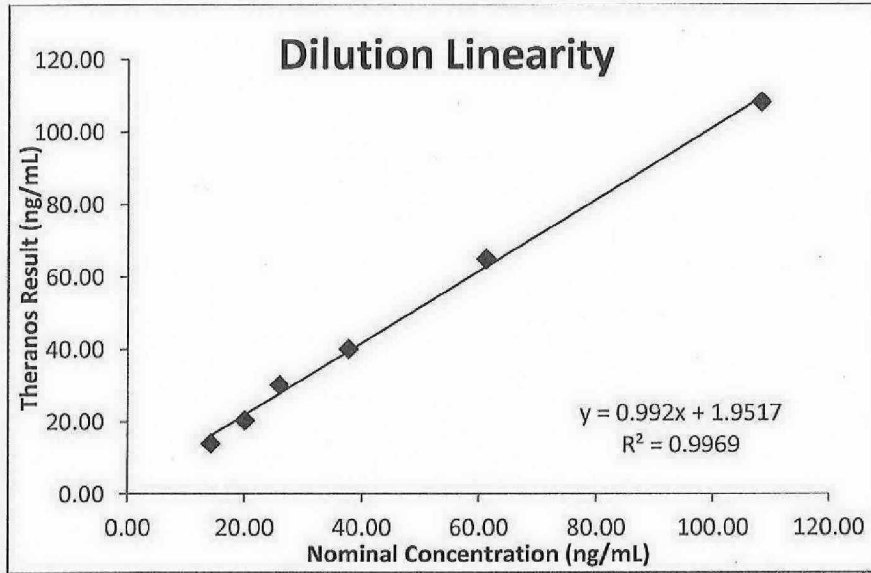
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Figure 13: Dilution Linearity Nominal v. Theranos Result




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. The reference range for 25OH Vitamin D per the Diasorin manual is 9.3-47.9 ng/mL. 30 unique patients donated 2 venous tubes of blood and 3 fingerstick samples. Of the 30 samples tested 1 was above the reference range and 1 tested below the reference range.


Table 12: Venous v. Fingerstick Dark Tip and Outlier Exclusion Data

Sample Type	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge				Concentration		
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Tip 3
Venous 1	31447	36649	31447	36649	34048	11%	30875	14%	29299	13%	40.58	31.45	
Venous 2	26759	28645	26759	28645	27702	5%					51.0		
Finger 1	25172	23769	25172	23769	24470	4%	28248	13%	32159	15%	55.0		
Finger 2	29067	32710	29067	32710	30888	8%					45.0		
Finger 3	32143	26629	32143	26629	29386	13%					39.0		
Venous 1	37140	35776	37140	35776	36458	3%	34751	9%	32159	15%	30.70	32.83	34.77
Venous 2	31338	7	31338	DARK	31338						40.8		
Finger 1	8209	38626		38626	38626		30603	18%	32159	15%			
Finger 2	23846	27911	23846	27911	25879	11%					59.3		
Finger 3	30569	32064	30569	32064	31317	3%					42.3		
Venous 1	37703	38040	37703	38040	37871	1%	35514	8%	32676	16%	29.86	29.36	33.47
Venous 2	32897	33416	32897	33416	33157	1%					37.80	36.85	

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Finger 1	27	31	DARK	DARK														
Finger 2	26307	25380	26307	25380	25843	3%	29838	19%				52.36	54.88	45.18				
Finger 3	37673	29992	37673	29992	33832	16%						29.90	43.58					
Venous 1	41160	39110	41160	39110	40135	4%						25.11						
Venous 2	22676	25341	22676	25341	24008	8%						63.14						
Venous 1	41307	43185	41307	43185	42246	3%						24.92						
Venous 2	42847	43328	42847	43328	43088	1%						23.05						
Finger 1			DARK	DARK														
Finger 2	35040	34348	35040	34348	34694	1%	35892	7%				34.04	35.21	32.82				
Finger 3	34368	39811	34368	39811	37089	10%						35.18	26.88					
Venous 1	43226	40670	43226	40670	41948	4%						22.58	25.74					
Venous 2	31736	31961	31736	31961	31848	0%						40.01	39.57	33.59				
Venous 1	35581	39247	35581	39247	37414	7%						33.14	27.65					
Venous 2	32234	31256	32234	31256	31745	2%						39.05	40.96					
Finger 1	32574	35670	32574	35670	34122	6%						38.40	33.00					
Finger 2	32843	33972	32843	33972	33408	2%						37.90	35.86	39.26				
Finger 3	28576	29958	28576	29958	29267	3%						46.74	43.66					
Venous 1	58686	68469	58686	68469	63578	11%						8.84	OORL					
Venous 2	48071	58074	48071	58074	53073	13%						17.40	9.25	10.34				
Venous 1	57550	58107	57550	58107	57829	1%						9.60	9.22					
Venous 2	58263	58495	58263	58495	58379	0%						9.12	8.96					
Finger 1	6	78	DARK	DARK														
Finger 2	37412	38885	37412	38885	38148	3%						30.29	28.15	24.86				
Finger 3	42719	47639	42719	47639	45179	8%						23.18	17.82					
Venous 1	51090	53594	51090	53594	52342	3%						14.62	12.54					
Venous 2	43854	39862	43854	39862	41858	7%						21.85	26.81	20.76				
Venous 1	44438	42935	44438	42935	43687	2%						21.19	22.92					
Venous 2	44913	40859	44913	40859	42886	7%						20.67						
Finger 1	41086	40755	41086	40755	40921	1%						25.20						
Finger 2	41249	42437	41249	42437	41843	2%						24.99						
Finger 3	51056	51563	51056	51563	51309	1%						14.65						
Venous 1	25080	28107	25080	28107	26594	8%						55.72	47.84					
Venous 2	38518	37871	38518	37871	38195	1%						28.67	29.61	35.56				
Venous 1	38703	37839	38703	37839	38271	2%						28.41	29.65					
Venous 2	10890	38258		38258	38258								29.04					
Finger 1	26729	19	26729	DARK	26729							51.25						
Finger 2	25644	26445	25644	26445	26044	2%						54.15	51.99	50.37				
Finger 3	27489	29299	27489	29299	28394	5%						49.34	45.10					
Venous 1	26263	1	26263	DARK	26263							52.47						
Venous 2	37786	34503	37786	34503	36145	6%						29.73	34.94	39.66				
Venous 1	33710	31280	33710	31280	32495	5%						36.33	40.91					
Venous 2	32244	29697	32244	29697	30971	6%						39.03	44.22					
Finger 1	31949	33538	31949	33538	32744	3%						39.59	36.63					
Finger 2	32667	34531	32667	34531	33599	4%						38.23	34.90	40.05				
Finger 3	29288	28976	29288	28976	29132	1%						45.12	45.82					
Venous 1	33269	35983	33269	35983	34626	6%						37.12	32.50	41.71				
Venous 2	27756	27810	27756	27810	27783	0%						48.69	48.56					
Finger 1	34417	30940	34417	30940	32679	8%						35.09	41.60	48.06				


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Finger 2	28173	28780	28173	28780	28477	2%					47.6			
Finger 3	23278	24794	23278	24794	24036	4%					61.1			
Venous 1	31698	34983	31698	34983	33341	7%	29970	14%			40.08	34.13		44.41
Venous 2	27821	25379	27821	25379	26600	6%					48.53	54.88		
Finger 1	39286	40113	39286	40113	39700	1%			32958	16%	27.59	26.47		
Finger 2	36330	36641	36330	36641	36486	1%	34950	15%			31.95	31.46		35.12
Finger 3	27476	29857	27476	29857	28666	6%					49.37	43.88		
Venous 1	29552	31065	29552	31065	30308	4%					44.54	41.35		38.23
Venous 2	34133	36636	34133	36636	35384	5%	32846	10%			35.58	31.47		
Finger 1	24115	25078	24115	25078	24597	3%			29819	14%	58.56	55.73		
Finger 2	26108	33031	26108	33031	29570	17%	27800	12%			52.89	37.55		49.21
Finger 3	29935	28536	29935	28536	29235	3%					43.71	46.84		
Venous 1	26903	31324												
Venous 2	57423	52159	57423	52159	54791	7%					9.69	13.71		11.15
Venous 1	56012	57621	56012	57621	56816	2%					10.70	9.56		
Venous 2	51251	58768	51251	58768	55009	10%			49940	14%	14.48	8.78		
Finger 1	43548	43942	43548	43942	43745	1%					22.20	21.75		
Finger 2	39030	43045	39030	43045	41037	7%	44340	10%			27.95	22.79		21.62
Finger 3	44412	52064	44412	52064	48238	11%					21.22	13.79		
Venous 1	33225	33950	33225	33950	33587	2%					37.20	35.90		
Venous 2	22927	23959												
Venous 1	36451	42338	36451	42338	39394	11%	37046	9%			31.76	23.64		31.15
Venous 2	37649	38665	37649	38665	38157	2%			34573	13%	29.93	28.4		
Finger 1	16882	19901												
Finger 2	33823	33488	33823	33488	33656	1%	30864	11%			30.0	28.0		27.19
Finger 3	27337	28807	27337	28807	28072	4%								
Venous 1	24962	4	24962	DARK	24962									
Venous 2	16047	17043												
Venous 1	33501	34644	33501	34644	34072	2%								
Venous 2	33220	38697	33220	38697	35959	11%			29250	18%				
Finger 1	25261	28294												
Finger 2	21048	23505	21048	23505	22277	8%	26122	13%						
Finger 3	28684	29938	28684	29938	29311	3%					40.0	35.0		
Venous 1	30493	37550	30493	37550	34021	15%					42.5	34.0		
Venous 2	30602	27226	30602	27226	28914	8%	31468	14%			42.30	34.0		
Finger 1	20214	22823		22823	22823									
Finger 2	26925	34386	26925	34386	30655	17%	29594	16%	30427	15%	50.75	45.4		45.65
Finger 3	29883	33952	29883	33952	31918	9%					43.82	35.90		
Venous 1	32047	34963	32047	34963	33505	6%					39.40	34.17		
Venous 2	43067	46620	43067	46620	44844	6%			38705	12%	22.77	18.85		28.94
Venous 1	37229	39206	37229	39206	38218	4%					30.56	27.70		
Venous 2	38082	38425	38082	38425	38254	1%			39885	12%	29.30	28.80		
Finger 1	36342	39070	36342	39070	37706	5%					31.93	27.89		
Finger 2	40740	6	40740	DARK	40740		41772	11%			25.65			24.78
Finger 3	44314	48393	44314	48393	46354	6%					21.33	17.09		


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Venous 1	44355	46789	44355	46789	45572	4%	44411	5%	42674	12%	21.28	18.68	21.31
Venous 2	45082	41419	45082	41419	43251	6%					20.48	24.78	
Finger 1	4	58	DARK	DARK									
Finger 2	35341	34386	35341	34386	34863	2%	40936	17%			33.54	35.14	26.40
Finger 3	46269	47750	46269	47750	47009	2%					19.22	17.71	
Venous 1	34381	34942	34381	34942	34662	1%	34531	1%	35679	9%	35.15	34.20	34.90
Venous 2	26252	34269		34269	34269						36.65	33.33	
Finger 1	33526	35470	33526	35470	34498	4%	36253	10%			27.72	23.78	32.46
Finger 2	39197	42221	39197	42221	40709	5%					38.48	34.82	
Finger 3	32530	34577	32530	34577	33554	4%					18.70	15.90	
Venous 1	46766	49661	46766	49661	48213	4%	54027	13%	49229	16%	8.54	OORL	13.95
Venous 2	59143	65710	59143	65710	62426	7%					10.41	8.79	
Venous 1	56410	58762	56410	58762	57586	3%					19.80	15.54	
Venous 2	45713	50051	45713	50051	47882	6%					20.72	23.12	
Finger 1	44867	42770	44867	42770	43819	3%	42832	4%			20.54	23.97	23.11
Finger 2	45029	42068	45029	42068	43549	5%					25.67	24.64	
Finger 3	40724	41532	40724	41532	41128	1%					8.13	8.32	15.77
Venous 1	59769	59483	59769	59483	59626	0%	49802	23%	47257	21%	27.78	25.57	20.47
Venous 2	39153	40804	39153	40804	39978	3%					17.49	11.87	
Finger 1	47980	54446	47980	54446	51213	9%	44713	19%			23.46	35.93	
Finger 2	91934	101333									28.32	33.18	39.74
Finger 3	42491	33935	42491	33935	338213	16%					58.55	45.65	
Venous 1	38767	35562	38767	35562	37164	6%	31875	21%	37122	22%	39.07	38.62	25.71
Venous 2	24120	29053	24120	29053	26587	13%					14.65	18.80	
Venous 1	32222	32459	32222	32459	32341	1%	40620	19%			23.40	28.31	
Venous 2	51059	46675	51059	46675	48867	6%					35.90		31.68
Finger 1	42535	38773	42535	38773	40654	7%					33.08	26.06	
Finger 2	33952	6	33952	DARK	33952		36665	9%	33740	12%	28.68	38.16	39.46
Venous 1	35623	40421	35623	40421	38022	9%					47.82	44.81	
Venous 2	38516	32701	38516	32701	35608	12%	32278	11%			40.14	37.17	
Finger 1	28417	29427	28417	29427	28772	3%					8.96	OORL	17.22
Finger 2	31668	33238	31668	33238	32453	3%					OORL	25.48	
Finger 3	31668	33238	31668	33238	32453	3%					OORL	8.55	
Venous 1	58500	63539	58500	63539	61020	6%	56312	19%	63024	18%	OORL	OORL	8.64
Venous 2	62338	40869	62338	40869	51604	29%					OORL	8.74	
Finger 1	66051	59128	66051	59128	62590	8%	67500	15%			OORL	OORL	
Finger 2	82318	78523	82318	78523	80421	3%					OORL	OORL	
Finger 3	60141	58836	60141	58836	59488	2%					9.23	OORL	9.48
Venous 1	58101	62629	58101	62629	60365	5%	59852	4%	51373	17%	9.74	OORL	
Venous 2	57358	61321	57358	61321	59339	5%					24.20	23.09	
Finger 1	41883	42793	41883	42793	42338	2%	45720	13%			27.50	21.32	20.37
Finger 2	39354	44321	39354	44321	41838	8%					13.89	12.20	
Finger 3	51945	54025	51945	54025	52985	3%					26.86	13.88	21.70
Venous 1	39820	51954	39820	51954	45887	19%	44370	12%	45220	11%	22.49	23.56	20.12
Venous 2	43302	42402	43302	42402	42852	1%					25.65	27.96	
Finger 1	40738	39021	40738	39021	39880	3%	45901	12%			15.71		
Finger 2	49865	4	49865	DARK	49865						15.16	16.14	
Finger 3	50478	49401	50478	49401	49940	2%							

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Sample Type	26976	26951	26976	26951	26964	0%	34379	25%	34809	17%	50.62	50.69	37.50					
Venous 2	43132	40458	43132	40458	41795	5%							22.69	26.02				
Finger 1	35918	37375	35918	37375	36647	3%	35096	12%			32.60	30.34	34.54					
Finger 2	37488	39880	37488	39880	38684	4%								30.17	26.79			
Finger 3	30249	29667	30249	29667	29958	1%								43.04	44.29			
Venous 1	28115	29633	28115	29633	28874	4%	34196	18%	31320	15%	47.82	44.36	36.68					
Venous 2	39513	39524	39513	39524	39518	0%										27.28	27.27	
Finger 1	26703	29662	26703	29662	28183	7%	29403	8%					51.32	44.30	45.15			
Finger 2	27633	29437	27633	29437	28535	4%										48.99	44.79	
Finger 3	33768	29217	33768	29217	31492	10%										36.22	45.28	
Venous 1	4245	20322		20322	20322		20388	11%			18906	10%		71.70	71.94			
Venous 2	18211	22630	18211	22630	20420	15%										80.81	63.29	
Finger 1	17082	16209	17082	16209	16645	4%	18301	8%					86.39	91.11	80.74			
Finger 2	20108	19371	20108	19371	19740	3%										72.55	75.61	
Finger 3	18627	18407	18627	18407	18517	1%										78.89	79.90	
Venous 1	5087	29153		29153	29153		31633	8%	29802	9%				45.43	40.42			
Venous 2	31656	34089	31656	34089	32873	5%										40.17	35.66	
Finger 1	32357	31299	32357	31299	31828	2%	28886	8%					38.81	40.87	46.32			
Finger 2	26840	27879	26840	27879	27360	3%										50.97	48.39	
Finger 3	28320	26621	28320	26621	27471	4%										47.34	51.53	

Table 13: Fingertstick v. Venous Blood Summary

Sample ID	Sample Type	Theranos Result [ng/mL]	%CV	% difference
Patient 1	Venous 1	42.43	20%	12%
	Finger 1	48.26	18%	
Patient 2	Venous 1	34.77	15%	20%
	Finger 1	43.59	26%	
Patient 3	Venous 1	33.47	13%	26%
	Finger 1	45.18	25%	
Patient 4	Venous 1	24.33	8%	26%
	Finger 1	32.82	12%	
Patient 5	Venous 1	33.59	22%	14%
	Finger 1	39.26	13%	
Patient 6	Venous 1	10.34	30%	58%
	Finger 1	24.86	22%	
Patient 7	Venous 1	20.76	24%	3%
	Finger 1	21.37	25%	
Patient 8	Venous 1	35.56	32%	29%
	Finger 1	50.37	7%	
Patient 9	Venous 1	39.66	18%	1%
	Finger 1	40.05	11%	
Patient 10	Venous 1	41.71	20%	13%
	Finger 1	48.06	20%	
Patient 11	Venous 1	44.41	21%	-26%
	Finger 1	35.12	27%	
Patient 12	Venous 1	38.23	15%	22%

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	Finger 1	49.21	16%	
Patient 13	Venous 1	11.15	21%	48%
	Finger 1	21.62	21%	
Patient 14	Venous 1	31.15	16%	26%
	Finger 1	42.19	16%	
Patient 15	Venous 1	38.62	27%	28%
	Finger 1	53.69	18%	
Patient 16	Venous 1	41.22	20%	10%
	Finger 1	45.65	25%	
Patient 17	Venous 1	28.94	22%	-17%
	Finger 1	24.78	23%	
Patient 18	Venous 1	21.31	12%	19%
	Finger 1	26.40	35%	
Patient 19	Venous 1	34.90	2%	-8%
	Finger 1	32.46	17%	
Patient 20	Venous 1	13.95	34%	40%
	Finger 1	23.11	9%	
Patient 21	Venous 1	17.45	61%	21%
	Finger 1	22.19	46%	
Patient 22	Venous 1	41.42	33%	-28%
	Finger 1	32.35	24%	
Patient 23	Venous 1	31.68	16%	20%
	Finger 1	39.46	17%	
Patient 24	Venous 1	17.22	68%	-99%
	Finger 1	8.64	2%	
Patient 25	Venous 1	9.48	4%	53%
	Finger 1	20.37	30%	
Patient 26	Venous 1	21.70	26%	-8%
	Finger 1	20.12	31%	
Patient 27	Venous 1	37.50	41%	-9%
	Finger 1	34.54	21%	
Patient 28	Venous 1	36.68	30%	19%
	Finger 1	45.15	11%	
Patient 29	Venous 1	71.94	12%	11%
	Finger 1	80.74	9%	
Patient 30	Venous 1	40.42	12%	13%
	Finger 1	46.32	11%	

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9 ANTICOAGULANT COMPARISON

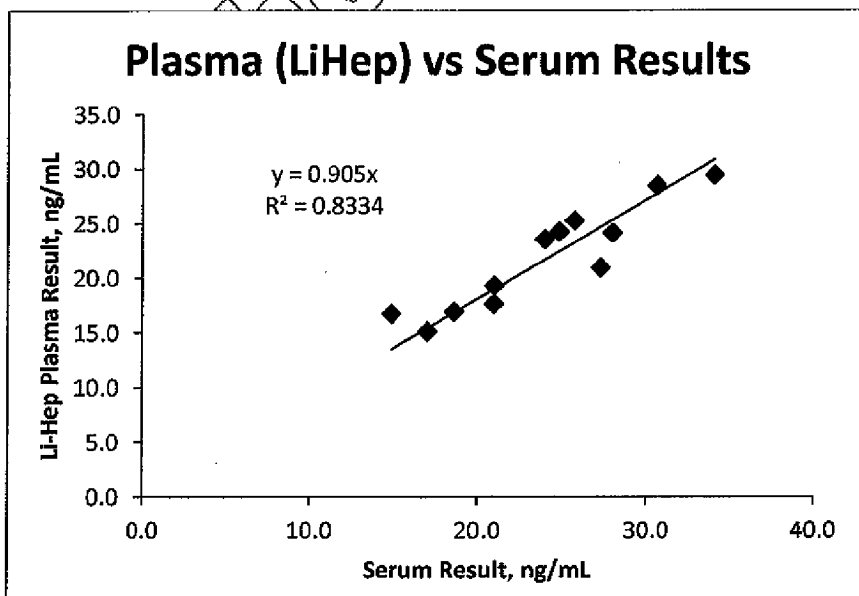
Data for the anticoagulant and serum testing was obtained from the development report.

To test for a matrix effect due to serum or plasma, matched blood samples were collected from 12 donors in lithium heparin tubes and in serum tubes and centrifuged to obtain plasma and serum respectively. The matched samples were tested in the Theranos System and the results compared, based on the assay buffer calibration curve. There was no significant difference between serum and plasma results, either matrix is suitable for the Theranos 25-Hydroxyvitamin D assay.

Table 14: Li Heparin v. Serum

Sample #	Gender	Plasma		Serum		% Recovery in Plasma vs Serum
		Mean Conc	CV %	Mean Conc	CV %	
01	M	16.7	7.3	14.9	8.3	112
02	F	20.9	6.3	27.3	8.1	77
03	M	25.2	1.9	25.7	8.1	98
04	M	17.6	9.5	21.0	10.9	84
05	M	24.1	3.4	28.0	2.2	86
06	M	29.4	3.6	34.1	2.5	86
07	F	23.5	8.2	23.9	6.7	98
08	M	28.5	5.6	30.6	6.9	93
09	M	24.2	5.2	24.8	95.0	98
10	M	19.3	3.7	21.0	8.9	92
11	F	16.9	5.8	18.6	5.3	91
12	M	13.1	3.6	17.1	10.7	89

Figure 14: Li Heparin v. Serum



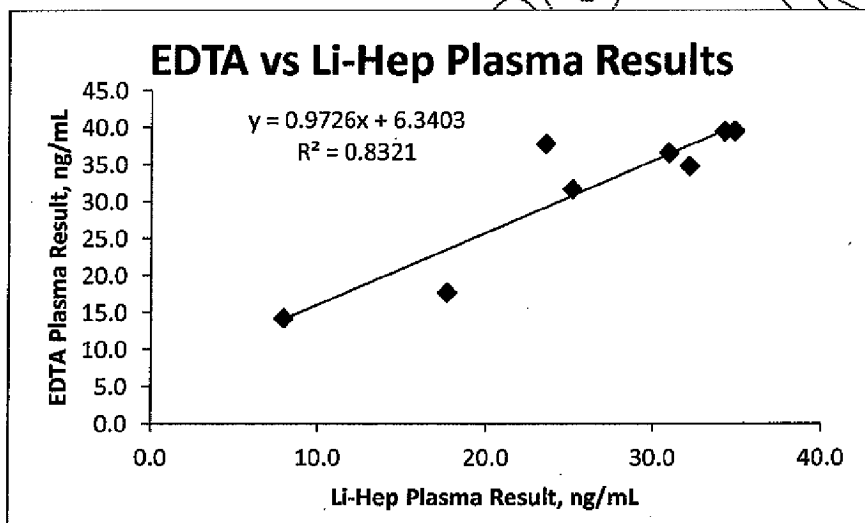
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The Theranos System is capable of preparing plasma from blood treated with either EDTA or lithium heparin as an anticoagulant in the collection system. To test for a bias due to anticoagulant, matched samples were collected from 10 donors in EDTA and in lithium heparin tubes and centrifuged to prepare plasma to test on the Theranos System. There was no significant difference between the lithium heparin and EDTA results.

Table 15: Li Heparin v. EDTA Plasma

Sample ID	Gender	EDTA Plasma		Li-Hep Plasma		% Recovery in EDTA vs Li-Hep
		Mean Conc	CV %	Mean Conc	CV %	
03	M	31.7	3.8	25.1	7.7	126
04	M	39.5	3.1	34.9	2.9	113
05	F	14.2	9.0	8.0	6.2	178
06	F	17.7	4.2	17.7	7.2	100
07	F	34.8	7.9	32.2	5.9	108
08	F	39.5	11.0	34.3	5.8	115
09	M	37.8	6.3	23.5	8.6	161
10	M	36.5	3.8	30.9	8.6	118

Figure 15: Li Heparin v. EDTA



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10 ANALYTICAL SENSITIVITY

To test the analytical sensitivity, 60 replicates of a blank sample and 60 replicates at the LLOQ were tested. To determine the lowest detectable dilution level, 20 replicates of LLOQ dilutions were tested. At 1/4 LLOQ, all but one point was out of the assay range.

Table 16: 1/2xLLOQ 1/4xLLOQ

1/2 LLOQ					
RLUs		Calculated From Mean	Calculated		
Mean	%CV		Mean	%CV	
70653	38%	OORL	18.59	28%	
All Tips		Inter-Cartridge	Conc. (ng/mL)		
System Error					
System Error					
System Error					
49621	50845	50233	2%	19.72	18.80
44827	44234	44531	1%	23.70	24.24
43680	51083	47382	11%	24.75	18.63
49033	51754	50394	4%	20.18	18.14
58693	56637	57665	3%	13.58	14.85
41213	48107	44660	11%	27.49	28.91
103857	20	51939	141%	OORL	OORL
62103	70806	66455	9%	11.80	OORL
System Error					
77118	76666	76892	0%	OORL	OORL
101658	100896	101277	1%	OORL	OORL
87529	100274	93902	10%	OORL	OORL
108889	94046	101468	10%	OORL	OORL
System Error					
63557	61852	62705	2%	10.79	11.74
115509	74422	94966	31%	OORL	OORL
109232	106992	108112	1%	OORL	OORL
74622	81134	77878	6%	OORL	OORL

1/4 LLOQ					
RLUs		Calculated From Mean	Calculated		
Mean	%CV		Mean	%CV	
92340	17%	OORL	71.30		
All Tips		Inter-Cartridge	Conc. (ng/mL)		
Tip1	Tip2	Mean	%CV	Tip 1	Tip 2
System Error					
93085	102348	97717	7%	OORL	OORL
103860	111975	107921	5%	OORL	OORL
19669	93134	56402	92%	71.30	OORL
84897	87504	86201	2%	OORL	OORL
97025	97021	97023	0%	OORL	OORL
100874	94633	97754	5%	OORL	OORL
87356	96663	92010	7%	OORL	OORL
91569	93013	91791	0%	OORL	OORL
82191	79811	80706	2%	OORL	OORL
97534	107179	102357	7%	OORL	OORL
98694	88707	93701	8%	OORL	OORL
85023	96049	90536	9%	OORL	OORL
103790	98369	101080	4%	OORL	OORL
108630	96627	102629	8%	OORL	OORL
72244	85686	78965	12%	OORL	OORL
88713	86659	87686	2%	OORL	OORL
107139	102862	105001	3%	OORL	OORL
69663	78511	74087	8%	OORL	OORL
111652	110160	110906	1%	OORL	OORL



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Table 17: Blank and Lowest Detectable Sensitivity

Blank					
RLUs		Calculated From		Calculated	
Mean	%CV	Mean	%CV	Mean	%CV
94922	30%	OORL		66.74	100%
All Tips		Inter-Cartridge		Conc. (ng/mL)	
Tip1	Tip2	Mean	%CV	Tip 1	Tip 2
108722	104512	106617	3%	OORL	OORL
84051	87185	85618	3%	OORL	OORL
94925	99265	97095	3%	OORL	OORL
116732	120449	118591	2%	OORL	OORL
118037	102725	110381	10%	OORL	OORL
12162	99432	55797	111%	127.54	OORL
84134	92076	88105	6%	OORL	OORL
10813	80617	45715	108%	148.67	OORL
115663	120069	117866	3%	OORL	OORL
84461	104125	94293	15%	OORL	OORL
101087	94379	97733	5%	OORL	OORL
107517	118571	113044	7%	OORL	OORL
81021	103049	92035	17%	OORL	OORL
94242	95708	94975	1%	OORL	OORL
72625	98612	85619	21%	OORL	OORL
31875	108445	70160	77%	39.30	OORL
74344	83641	78993	8%	OORL	OORL
118778	132822	125800	8%	OORL	OORL
101964	112222	107093	7%	OORL	OORL
87441	95328	91385	6%	OORL	OORL
102121	110890	106506	6%	OORL	OORL
94427	94448	94438	0%	OORL	OORL
111368	113617	112493	1%	OORL	OORL
101395	100290	100843	1%	OORL	OORL
90172	90654	90413	0%	OORL	OORL
104795	4	52400	141%	OORL	OORH
94686	98882	96784	3%	OORL	OORL
88584	92278	90431	3%	OORL	OORL
107795	8	53902	141%	OORL	OORH
93437	96886	95162	3%	OORL	OORL
108112	108011	108062	0%	OORL	OORL
104712	103381	104047	1%	OORL	OORL
113548	89688	101618	17%	OORL	OORL
49	57	53	11%	OORH	OORH
162888	167569	165229	2%	OORL	OORL
92888	79118	86003	11%	OORL	OORL
82689	92393	87541	8%	OORL	OORL
114127	114653	114390	0%	OORL	OORL
119697	111631	115664	5%	OORL	OORL
93464	88656	91060	4%	OORL	OORL

LLOQ (10ng/mL)					
RLUs		Calculated From		Calculated	
Mean	%CV	Mean	%CV	Mean	%CV
71704	31%	OORL		16.53	77%
All Tips		Inter-Cartridge		Conc. (ng/mL)	
Tip1	Tip2	Mean	%CV	Tip 1	Tip 2
87059	86261	86660	1%	OORL	OORL
82704	91093	86899	7%	OORL	OORL
90657	95670	93164	4%	OORL	OORL
71660	71311	71486	0%	OORL	OORL
65141	79325	72233	14%	9.93	OORL
74504	80315	77408	5%	OORL	OORL
80691	87962	84327	6%	OORL	OORL
63937	69325	66631	6%	10.58	OORL
75268	80981	78125	5%	OORL	OORL
50559	84065	67312	35%	19.01	OORL
80285	77623	78954	2%	OORL	OORL
70664	72019	71342	1%	OORL	OORL
18227	73221	45724	85%	78.02	OORL
88461	98217	93339	7%	OORL	OORL
71194	71032	71113	0%	OORL	OORL
87991	65917	76654	21%	OORL	9.83
77966	66414	72190	11%	OORL	9.25
43159	78248	60704	41%	25.25	OORL
76217	75119	75668	1%	OORL	OORL
97061	101253	99157	3%	OORL	OORL
71564	75377	73471	4%	OORL	OORL
108137	100656	104397	5%	OORL	OORL
69260	70145	69703	1%	OORL	OORL
84874	83639	84257	1%	OORL	OORL
79778	23	39901	141%	OORL	OORH
51804	52446	52125	1%	18.10	17.65
55883	68832	62358	15%	15.33	OORL
74373	75325	74849	1%	OORL	OORL
91929	77670	84800	12%	OORL	OORL
76801	62396	69599	15%	OORL	11.43
System Error					
51721	52510	52116	1%	18.16	17.60
65509	61439	63474	5%	9.73	11.97
66536	64620	65578	2%	9.19	10.21
70697	5	35351	141%	OORL	OORH
41202	41658	41430	1%	27.20	26.73
97260	5	48633	141%	OORL	OORH
77604	84544	81074	6%	OORL	OORL
75281	82478	78880	6%	OORL	OORL
98164	101522	99843	2%	OORL	OORL

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84463	79887	82175	4%	OORL	OORL
System Error					
79391	90246	84819	9%	OORL	OORL
99817	107187	103502	5%	OORL	OORL
96914	94066	95490	2%	OORL	OORL
108455	109609	109032	1%	OORL	OORL
106033	113262	109648	5%	OORL	OORL
109883	117186	113535	5%	OORL	OORL
115564	120635	118100	3%	OORL	OORL
108325	105004	106665	2%	OORL	OORL
95813	100491	98152	3%	OORL	OORL
86115	83919	85017	2%	OORL	OORL
98109	106615	102362	6%	OORL	OORL
93723	6	46865	141%	OORL	OORH
98357	106492	102425	6%	OORL	OORL
133550	117310	125430	9%	OORL	OORL
103547	104901	104224	1%	OORL	OORL
67409	66043	66726	1%	8.73	9.45
77313	86247	81780	8%	OORL	OORL
117170	123835	120503	4%	OORL	OORL


57154	57515	57335	0%	14.53	14.30
83790	12	41901	141%	OORL	OORH
85271	87502	86387	2%	OORL	OORL
103839	102245	103042	1%	OORL	OORL
48324	52931	50628	6%	20.73	17.31
75743	80023	77883	4%	OORL	OORL
76790	82234	79512	5%	OORL	OORL
82341	84422	83382	3%	OORL	OORL
88534	7	44271	141%	OORL	OORH
80312	88564	84438	7%	OORL	OORL
78767	79429	79098	1%	OORL	OORL
70402	76207	73350	6%	OORL	OORL
61367	66403	63886	6%	12.01	9.26
84478	86608	85543	2%	OORL	OORL
62298	63598	62948	1%	11.49	10.76
67705	1	33853	141%	8.58	OORH
61695	65099	63397	4%	11.83	9.95
91065	94673	92869	3%	OORL	OORL
70262	73111	71687	3%	OORL	OORL
87047	81377	84212	5%	OORL	OORL

11 INTERFERENCE

The recovery of analyte spiked into hemolyzed (500mg/dL Hemoglobin), icteric (40mg/dL), and lipemic (1000mg/dL Triglycerides) serum samples was evaluated on the Theranos System and compared to the predicate method. Lipemic samples containing triglycerides at 1000 mg/dl had very low recovery and should not be used for testing on the Theranos System.

Table 18: Interfering Substances

Nominal Value (ng/mL)	Hemolyzed sample (hemoglobin 500mg/dL)						Dexter Calculated Result						
	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Concentration			%CV	%Recovery
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean		
100	18297	17848	18297	17848	18073	2%	16607	10%	80.41	82.54	88.91	10%	89%
	14339	16313	14339	16313	15326	9%			102.69	90.53			
	15221	17624	15221	17624	16423	10%			96.95	83.64			
75	20099	20095	20099	20095	20097	0%	23732	18%	72.59	72.61	59.74	22%	80%
	26191	28543	26191	28543	27367	6%			52.66	46.82			
	4	224	DARK										
50	20972	20921	20972	20921	20947	0%	21541	4%	69.19	69.38	67.09	4%	134%
	22298	21424	22298	21424	21861	3%			64.42	67.51			
	20911	22718	20911	22718	21815	6%			69.42	63.00			
0	39883	35186	39883	35186	37535	9%	43546	25%	26.78	33.79	22.21	56%	
	32763	46	32763	DARK	32763				38.05				
	58300	51598	58300	51598	54949	9%			9.09	14.18			

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Icteric sample (Bilirubin 40mg/dL)							Dexter Calculated Result							
Nominal Value (ng/mL)	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Concentration			%CV	%Recovery	
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean			
100	14045	16014	14045	16014	15030	9%	15030	9%	104.73	92.22	98.15	9%	98%	
	14	14	DARK	DARK										
	185	5		DARK										
75	22006	25445	22006	25445	23726	10%	18945	29%	65.43	54.70	77.46	34%	103%	
	20680	13	20680	DARK	20680				70.30					
	12865	13728	12865	13728	13297	5%			113.61	107.00				
50	20065	19930	20065	19930	19998	0%	19747	23%	72.73	73.28	74.03	26%	148%	
	14762	15022	14762	15022	14892	1%			94.87	98.20				
	26855	21849	26855	21849	24352	15%			50.93	65.98				
0	27853	31788	27853	31788	29821	9%	32177	10%	48.45	39.91	39.15	17%		
			DARK	DARK										
	33725	35341	33725	35341	34533	3%			36.30	33.54				

Lipemic sample (Triglycerides 1000mg/dL)							Dexter Calculated Result							
Nominal Value (ng/mL)	All Tips		Dark Exclusion		Inter-Cartridge		Intra-Cartridge		Concentration			%CV	%Recovery	
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean			
100	29682	32942	29682	32942	31312	7%	30237	17%	44.25	37.72	43.06	26%	43%	
	23808	24944	23808	24944	24376	3%			59.50	56.11				
	33943	36102	33943	36102	35023	4%			35.91	32.31				
75	28990	33739	28990	33739	31365	11%	31094	17%	45.79	36.27	41.29	24%	55%	
	38788	48906	38788		38788				28.29					
	25981	27974	25981	27974	26978	5%			53.23	48.16				
50	32645	34156	32645	34156	33401	3%	36893	17%	38.27	35.54	31.07	28%	62%	
	32809	32449	32809	32449	32629	1%			37.96	38.64				
	46800	42501	46800	42501	44651	7%			18.67	23.44				
0	55411	10	55411	DARK	55411		53233	8%	11.14		12.83	26%		
	49310	3	49310	DARK	49310				16.22					
	50190	58021	50190	58021	54106	10%			15.42	9.28				

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Figure 16: Hemolyzed Sample

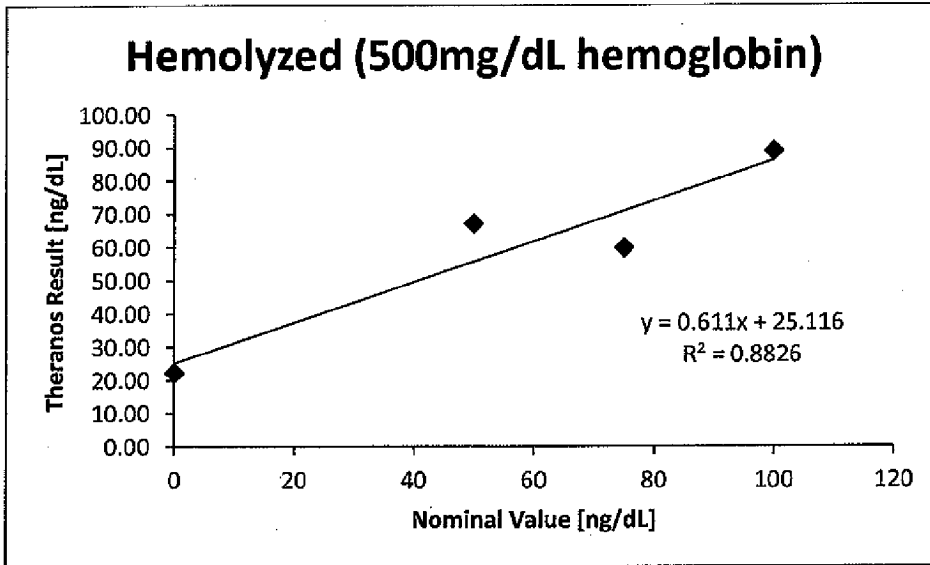
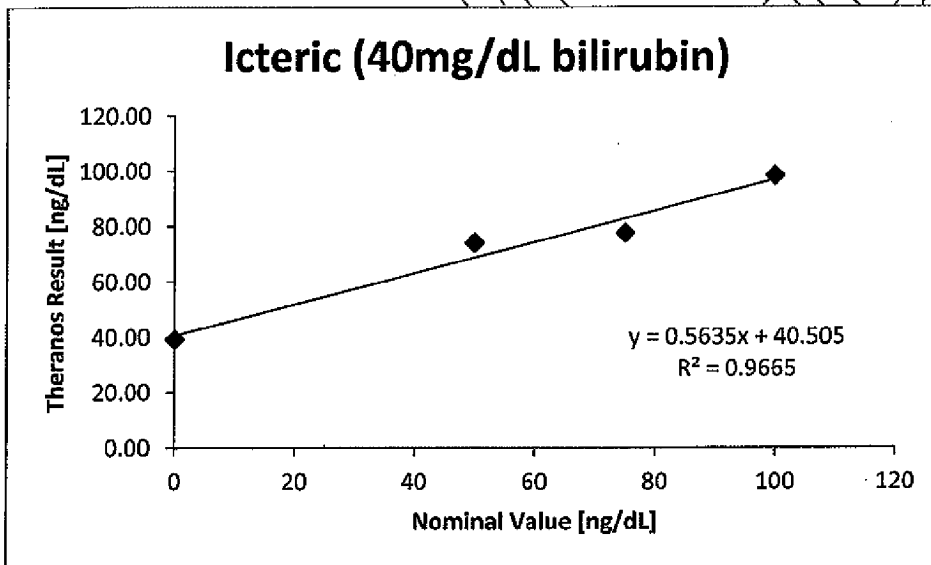
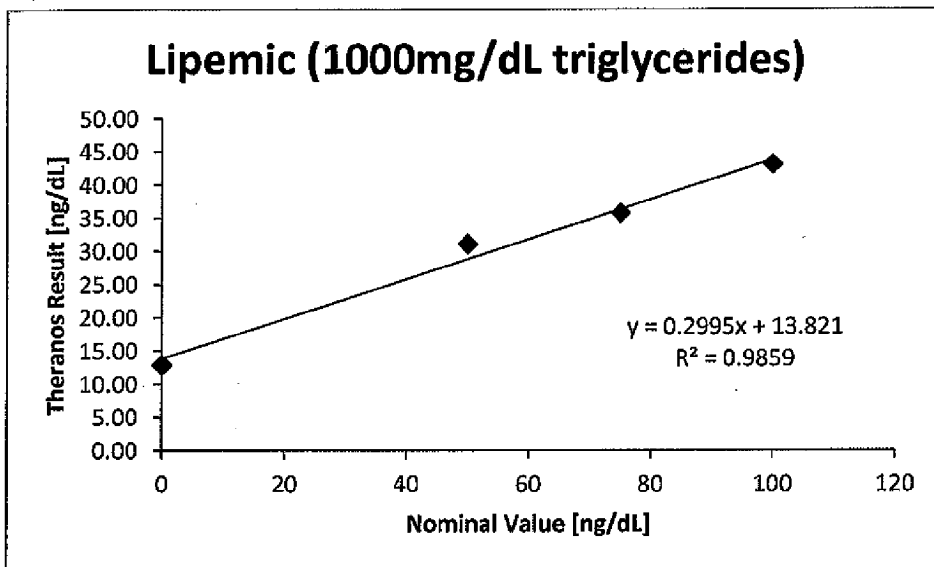


Figure 17: Icteric Sample



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Figure 18: Lipemic Sample



12 CROSSREACTIVITY

Information about cross-reacting analytes was obtained from the predicate method.

No cross reactivity was observed with Vitamin D3 (Cholecalciferol), Vitamin D2 (Ergocalciferol), or 24,25-DihydroxyVitamin D3. Cross reactivity was observed with 1 α ,25-DihydroxyVitamin D3, another hydroxylated metabolite of Vitamin D, however this is not a clinical concern because blood levels of 1 α ,25-Dihydroxy Vitamin D are in the order of 1000-fold lower than 25-Hydroxyvitamin D (in the pg/mL range). Cross reactivity with this metabolite is common in 25-Hydroxyvitamin D assays.

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Table 19: Cross-reactive Samples

Sample ID	Nominal Value (ng/mL)	All Tips		Inter-Cartridge		Intra-Cartridge		Concentration				%Recovery
		Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	
Control (25-HydroxyVitaminD3)	150	2286	2096	2191	6%	2432	12%	113.72	124.97	166.44	13%	71%
		2671	2676	2674	0%			96.48	96.29			
	100	2885	2997	2941	3%	2941	3%	89.09	85.67	87.34	3%	87%
		8	10									
	50	4131	4252	4192	2%	4044	5%	61.94	60.16	63.28	5%	127%
		3762	4030	3896	5%			68.05	63.50			
1 α ,25-DihydroxyVitamin D3	150	2237	2305	2271	2%	2352	4%	116.40	112.71	110.33	5%	74%
		2406	2458	2432	2%			107.67	105.26			
	100	3767	3642	3705	2%	3642	3%	67.96	70.31	70.31	3%	70%
		3503	3656	3580	3%			73.12	70.04			
	50	6290	9663	7977	30%	7804	18%	40.32	25.00	31.97	20%	64%
		7860	7402	7631	4%			31.72	33.89			
24,25-DihydroxyVitamin D3	150	23056	20942	21999	7%	22992	6%	4.52	6.48	4.58	30%	3%
		23922	24047	23985	0%			OORL	OORL			
	100	24146	23576	23861	2%	23735	2%	OORL	4.06	OORL	OORL	OORL
		23014	24203	23609	4%			4.36	OORL			
	50	24434	26115	25275	5%	25059	3%	OORL	OORL	OORL	OORL	OORL
		24405	25282	24844	2%			OORL	OORL			
Cholecalciferol	150	29411	28767	29089	2%	31607	10%	OORL	OORL	OORL	OORL	OORL
		32888	35361	34125	5%			OORL	OORL			
	100	30654	30304	30329	0%	30864	2%	OORL	OORL	OORL	OORL	OORL
		30878	31918	31398	2%			OORL	OORL			
	50	29796	30788	30292	2%	31046	4%	OORL	OORL	OORL	OORL	OORL
		31087	32512	31800	3%			OORL	OORL			
Ergocalciferol	150	32691	30025	31358	6%	30935	4%	OORL	OORL	OORL	OORL	OORL
		30116	30909	30513	2%			OORL	OORL			
	100	27321	27068	27195	1%	27040	1%	OORL	OORL	OORL	OORL	OORL
		26660	27111	26886	1%			OORL	OORL			
	50	29402	28751	29077	2%	30040	4%	OORL	OORL	OORL	OORL	OORL
		31297	30708	31003	1%			OORL	OORL			

13 STABILITY

The stability of serum samples 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 need to be processed within 12h if stored at 4 degrees and room temperature.


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Figure 19: Analyte Stability when stored at 4C

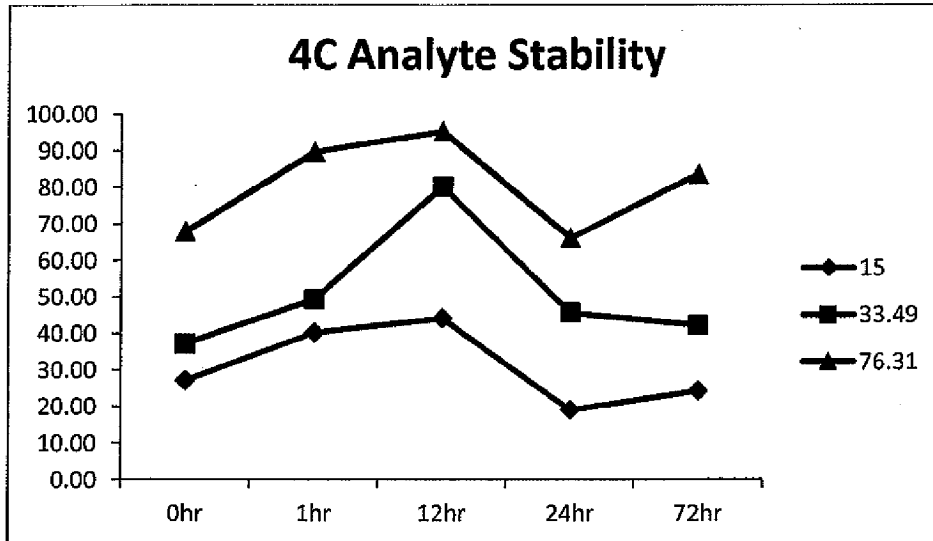
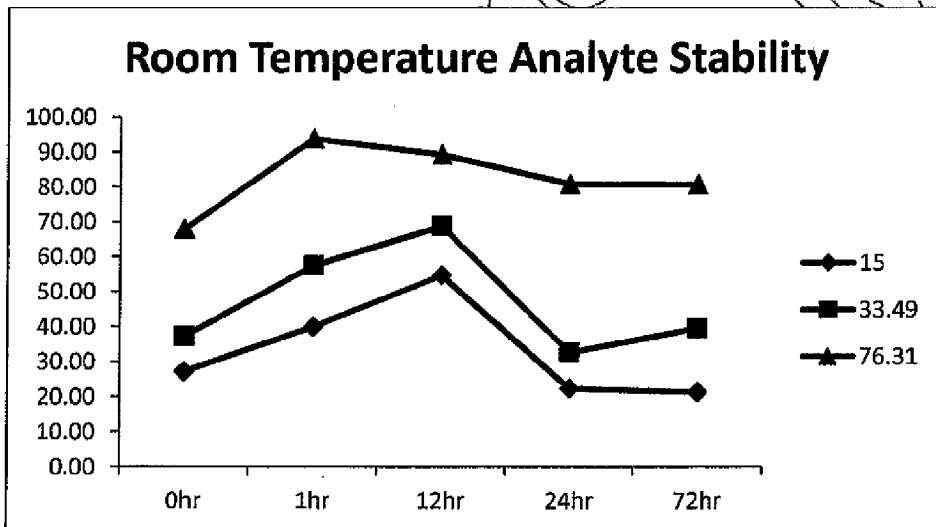


Figure 20: Analyte Stability when stored at Room Temperature

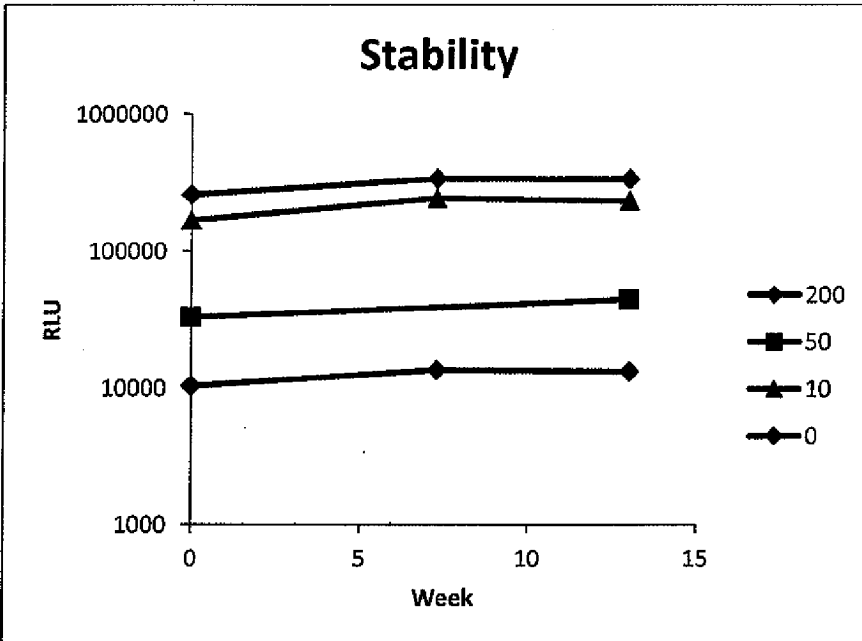


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14 Reagent Stability

Data from the development report were used to test the stability of the reagents stored at 4C. Reagents were stored outside of the cartridges and added immediately before testing. The reagents are stable at 4C for up to 13 weeks.

Figure 21: Stability of Reagents up to 13 weeks



theranos	25OHvitD Total Report	Document Number:
	Validation Document	Revision: A
		Effective Date:
25OHvitD Total ELISA Assay Validation Report on Edison 3.X Theranos System		

15 REFERENCES

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