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VITAMIN B12 ELISA Assay Validation Report on Edison 3.5 Theranos System

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1 ASSAY BACKGROUND

Vitamin B12 (cobalamin) is a nutrient essential for hematopoiesis and neurological function. It is involved in cellular metabolism, especially affecting DNA synthesis and regulation as well as fatty acid synthesis and energy production. Vitamin B12 or folate deficiencies are the main causes of megaloblastic anemia, and can potentially cause severe and irreversible damage to the brain and nervous system.

Circulating levels of vitamin B12 are generally a good reporter of tissue stores. Exceptions to this rule are uncommon, and include situations where levels of vitamin B12 transport proteins are abnormal. Circulating vitamin B12 levels can appear falsely low when the level of transcobalamin I (a physiologically inactive transport protein) is low. Alternatively, plasma vitamin B12 deficiency can appear normal or falsely high when transcobalamin II levels are low or where levels of inactive vitamin B12 transport proteins are high, as in chronic myelogenous leukemia. In this case, circulating folate levels would be used as a co-reporter; red blood cell folate levels are generally normal or elevated in vitamin B12 deficiencies, but low in this condition.

Vitamin B12 deficiency rarely occurs as a result of dietary lack of this vitamin. More commonly, it results from impaired absorption, e.g., after partial or total gastrectomy, or in pernicious anemia, a condition characterized by absence or near absence of intrinsic factor. Intrinsic factor is a glycoprotein necessary for the absorption of vitamin B12 in the small intestine. Roughly two thirds of all patients with pernicious anemia have blocking antibodies to intrinsic factor (IFbAb), and IFbAb are very rarely present in other situations. So, IFbAb detection provides a useful follow-up diagnostic in cases of vitamin B12 deficiency.

Common causes of high vitamin B12 levels include liver disease, myeloproliferative disease (with chronic myelogenous leukemia as a special case) and the use of multivitamin supplements.

1.1 Theranos System Specification

This assay is designed to detect Vitamin B12 in human EDTA plasma, Li Heparin Plasma, and Serum. The assay has a reportable range of 100 – 1000 pg/mL (Calibrator lot specific: current lot 103-1870 pg/mL). The calibrator values are verified by the SIEMENS Immulite 2000 Vitamin B12 test . pCTN sample stability shows that vitamin B12 in the pCTN is stable at 4C for 48 hours without being spun down. If samples are not analyzed within 48 hours of collection, samples should be spun down, and the plasma should be separated and stored at -20C or lower.

Table 1: Theranos Vitamin B12 Assay Specification

Assay	Theranos Vitamin B12 Assay
Reportable Range	100 - 1000 pg/mL
Accepted sample type	EDTA, Lithium Heparin and Serum
Sample Stability	pCTN sample stability shows that vitamin B12 in the pCTN is stable at 4C for 48 hours.

1.2 Reference Assay

The SIEMENS Immulite 2000 Vitamin B12 test was used as a predicate method. The reportable range is 150-1000 pg/mL. Hemolysis (packed red blood cells in concentration up to 417 mg/dL), lipemia (up to 4000 mg/dL triglycerides), and icterus (up to 200 mg/L bilirubin), do not affect assay precision.

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.

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 ± 20% (100 ± 25% at LLOQ and ULOQ standards) of their

nominal values, and a minimum of six unique standard concentrations must be within the assay range.

3.2 Calibrator preparation

Vitamin B12 assay calibrators were prepared by serially diluting vitamin B12 analyte stock into synthetic serum substitute which has no detectable B12. Each calibrator was analyzed by predicate method. Calibrators 4-7 were directly assigned the values from the predicate method. For Calibrator 1, a 5-fold dilution was made using predicate method kit diluent and tested. For Calibrators 2 and 3, 2-fold dilutions were made and tested. The reassigned were calculated using the corresponding dilution factor. Calibrator 8 was below the detection limit of the predicate method and was reassigned its value by dividing the Calibrator 7 value by its dilution factor.

Table 2: Calibrator preparation
Two sets of calibrators were prepared. Readers 1, 2 and 3 were calibrated using Calibrator set#1. Readers 4, 5 and 6 were calibrated using Calibrator set#2.

Calibrator set #1

Vitamin B12	nominal conc [pg/mL]	CLIA results [pg/mL]	Reassigned Value [pg/mL]
Calibrator 1	2000	327*	1635
Calibrator 2	1000	519*	1038
Calibrator 3	800	423*	846
Calibrator 4	500	471	471
Calibrator 5	400	333	333
Calibrator 6	300	264	264
Calibrator 7	200	185	185
Calibrator 8	100	< 150	92
Calibrator 9	0	< 150	0

*CLIA results for diluted samples. (Level 1: 5X Dilution; Level 2-3: 2X Dilution)

Calibrator set #2

Vitamin B12	nominal conc [pg/mL]	CLIA results [pg/mL]	Reassigned Value [pg/mL]
Calibrator 1	2000	374*	1870
Calibrator 2	1000	505*	1011
Calibrator 3	800	423*	846
Calibrator 4	500	505	505
Calibrator 5	400	390	390
Calibrator 6	300	280	280

Calibrator 7	200	207	207
Calibrator 8	100	< 150	103
Calibrator 9	0	< 150	0

*CLIA results for diluted samples. (Level 1: 5X Dilution; Level 2-3: 2X Dilution)

Calibration (Edison 3.5) For All Validation Experiments
All 6 readers were calibrated separately. Each device was calibrated by nozzle (tip). For all readers, precision and accuracy were within 100 ± 25% at LLOQ and ULOQ standards.

4 PRECISION

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

4.2 Precision study procedure

4.2.1 Precision was evaluated at three medical decision levels. A total of 45 runs at each decision level were performed.

4.2.2 Three devices were chosen to run the precision study. Each of the medical decision level was run 15 times on each device.

4.3 Precision Data and Summary

Table 3: Acceptable Performance

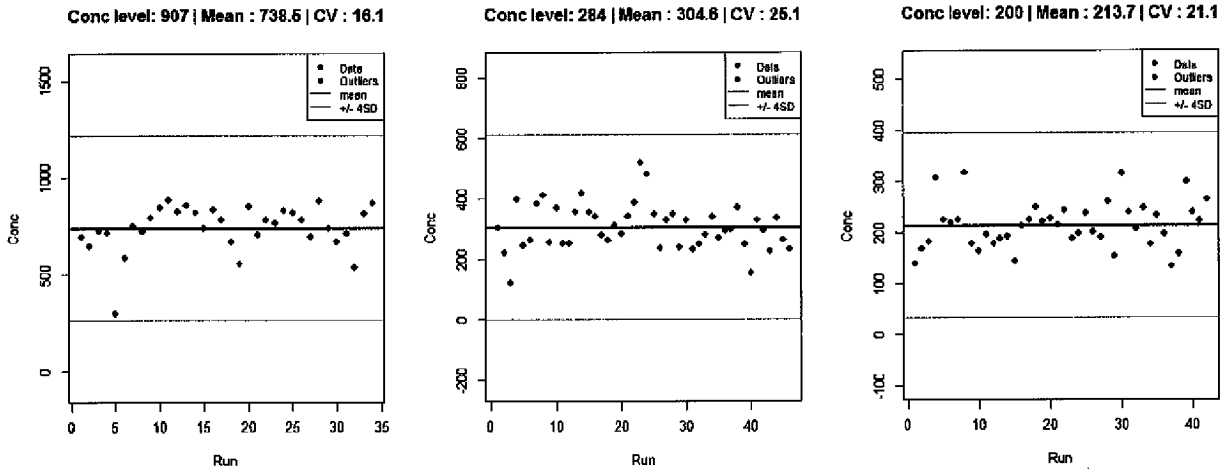
Decision Level	TAE(%)
1200 pg/mL	30 %
250 pg/mL	30 %
170 pg/mL	30 %

Table 4: Precision Summary

Medical Decision Levels	Vitamin B12 [pg/mL]		Precision CV	Allowable Bias
	Nominal value	CLIA Concentration		
1	900	907	16.1%	13.9%
2	250	284	25.1%	4.9%
3	170	200	21.1%	8.9%

Figure 1: Precision Plots

The plot shows the profile of predicted concentrations from precision study done over multiple runs.



5 ACCURACY/COMPARABILITY

5.1 To test the accuracy of the assay on the Theranos System, 112 unique patient samples were assayed using both the predicate method (Siemens Immulite) and the Theranos System. Of these 112 samples, 86 were within the reference range (193 - 982 pg/mL). 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.

5.2 Results: Deming's weighted linear regression was used to fit vitamin B12 method comparison data. Correlation between Theranos results and Immulite results was determined by equation of the fit, y=0.72x+57.7, Pearson's r = 0.818.

Table 5: Method comparison summary

Sample ID	CLIA Value, [B12 pg/mL]	Theranos Bias-Corrected Results, [B12 pg/mL]
Patient 1	192	192
Patient 2	152	135
Patient 3	237	213
Patient 4	>1000	OORH
Patient 5	168	OORL
Patient 6	161	OORL
Patient 7	<150	OORH
Patient 8	<150	OORL
Patient 9	176	273
Patient 10	< 150	OORL
Patient 11	< 150	175
Patient 12	< 150	170
Patient 13	187	206
Patient 14	<150	OORL
Patient 15	169	OORL
Patient 16	<150	OORL
Patient 17	166	OORL

Patient 18	179	OORL
Patient 19	<150	OORL
Patient 20	< 150	OORL
Patient 21	189	OORL
Patient 22	576	605
Patient 23	1043	829
Patient 24	416	486
Patient 25	708	483
Patient 26	516	282
Patient 27	604	591
Patient 28	423	295
Patient 29	480	252
Patient 30	>1000	OORH
Patient 31	477	385
Patient 32	474	475
Patient 33	240	OORL
Patient 34	582	268
Patient 35	517	245
Patient 36	269	312
Patient 37	349	415
Patient 38	852	442
Patient 39	549	506
Patient 40	512	172
Patient 41	608	449
Patient 42	915	648
Patient 43	1000	971
Patient 44	1140	OORH
Patient 45	412	305
Patient 46	332	OORH
Patient 47	955	807
Patient 48	947	734
Patient 49	489	226
Patient 50	379	374

Patient 51	470	525
Patient 52	245	287
Patient 53	203	242
Patient 54	194	127
Patient 55	214	176
Patient 56	707	807
Patient 57	925	508
Patient 58	295	194
Patient 59	371	241
Patient 60	858	466
Patient 61	732	784
Patient 62	503	382
Patient 63	274	215
Patient 64	280	174
Patient 65	1020	OORH
Patient 66	296	238
Patient 67	676	627
Patient 68	833	778
Patient 69	827	286
Patient 70	887	888
Patient 71	294	273
Patient 72	768	559
Patient 73	502	490
Patient 74	379	432
Patient 75	283	264
Patient 76	457	263
Patient 77	236	135
Patient 78	887	OORH
Patient 79	912	647
Patient 80	982.5	703
Patient 81	494	402
Patient 82	782	507
Patient 83	435	462

Patient 84	241	280
Patient 85	347	376
Patient 86	925	723
Patient 87	799	662
Patient 88	799	482
Patient 89	842	667
Patient 90	611	384
Patient 91	575	380
Patient 92	616	573
Patient 93	779	689
Patient 94	529	370
Patient 95	465	630
Patient 96	646	502
Patient 97	408	496
Patient 98	428	527
Patient 99	262	304
Patient 100	892	758
Patient 101	857	445
Patient 102	748	792
Patient 103	588	755
Patient 104	532	665
Patient 105	< 150	OORL
Patient 106	218	215
Patient 107	162	155
Patient 108	239	208
Patient 109	192	185
Patient 110	177	147
Patient 111	209	182
Patient 112	174	OORL

Figure 2: Scatter Plot using Deming's weighted regression after Bias correction– CLSI Guideline EP09-A2-IR Section 4.2

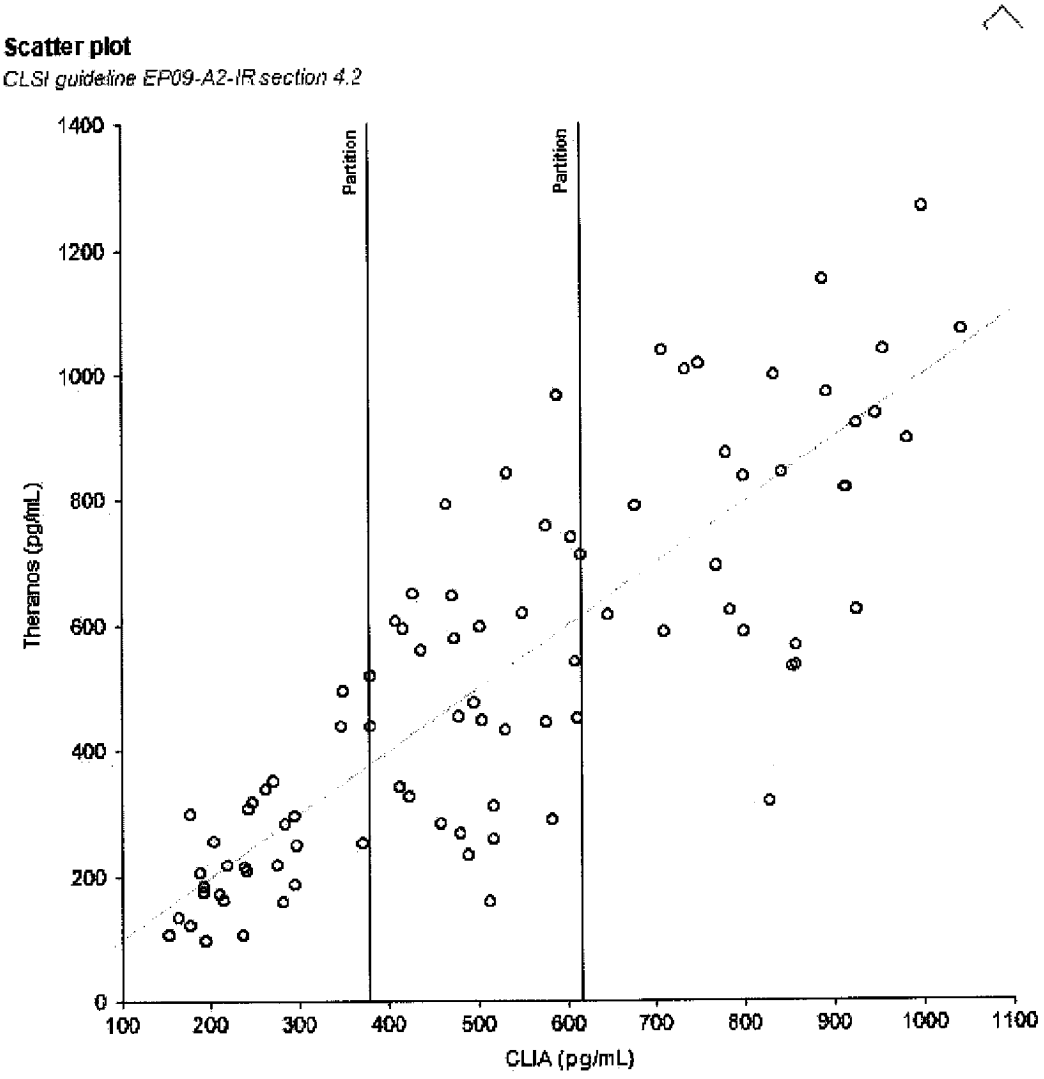
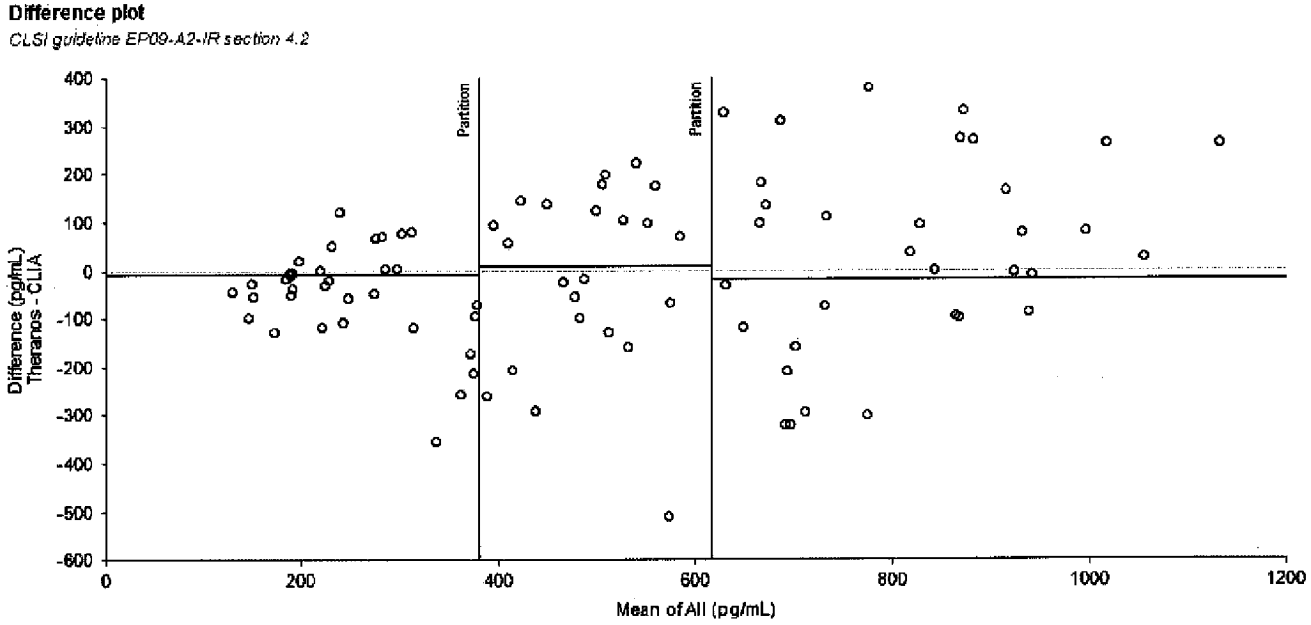


Figure 3: Difference Plot – CLSI Guideline EP09-A2-IR Section 4.2



Adequate range test

CLSI guideline EP09-A2-IR section 4.5

r	0.818

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
< 379	28	-7.71844850	74.84826093
≥ 379 and < 616	31	8.274774916	195.1620638
≥ 616	29	-18.1062514	208.6543054

Comparability

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
1	170.0000000	-7.71844850	14.14499126	-36.741574232 to 21.304	23.80000000
2	250.0000000	-7.71844850	14.14499126	-36.741574232 to 21.304	12.50000000
3	1200.000000	-18.1062514	38.74613197	-97.474104604 to 61.261	60.00000000

Difference is less than allowable bias: 14% upto 200pg/mL then 5%.

5.3 Validation of bias correction: In order to validate the bias correction derived in section 5.2, an independent set of samples was analyzed on Theranos as well as the predicate method. The objective of this study was to apply the bias correction on Theranos measurments, and then compare the mean bias between the two methods with the total allowable bias.

```
Total allowable bias = Total allowable error - Avg Imprecision
                     = 30% - 20.1%
                     = 9.9%
```

As seen in figure 10, after correction, the mean bias is 2.9%, with a 95% confidence interval around it of [-6.9%, 12%]. This bias is within the total allowable bias, this validating the bias correction. Out of the 30 samples used for this study, 4 samples were out of range on one of the two methods (but were consistent). Two samples had failed runs on the Theranos method which could not be repeated and 1 sample (Val10) repeatedly tested at a different value from the predicate method.

Figure 10 Validation of bias correction.

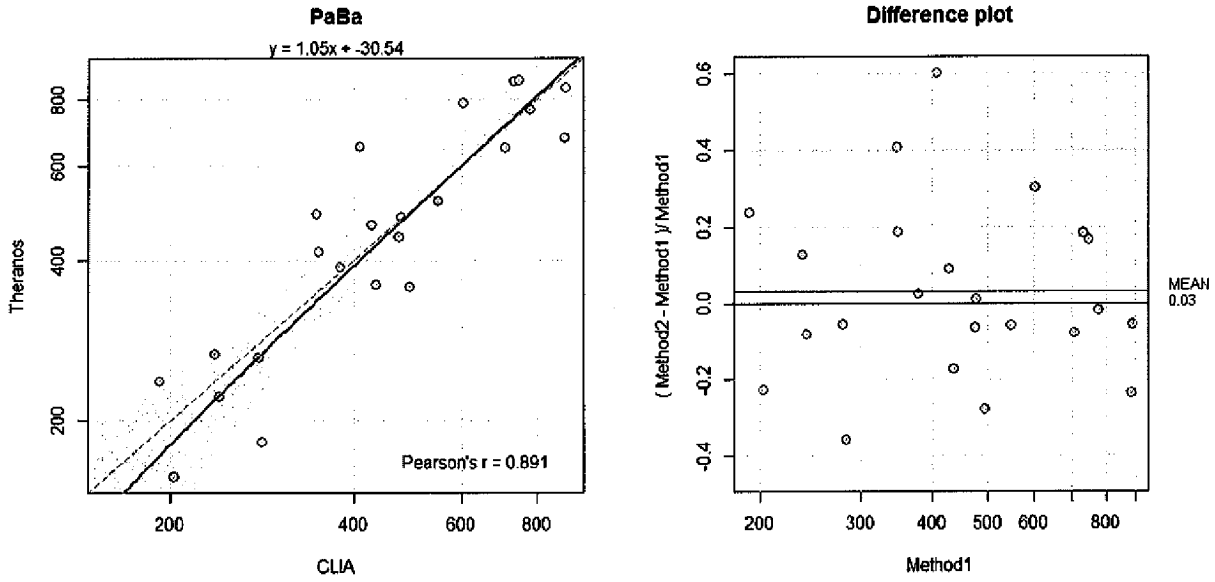


Table 12: Summary of Bias-Correction-Validation samples

SampleID	CLIA	Theranos	Comment
Val1	1000	OORH	Consistent
Val2	732	866.32	
Val3	283	182.21	
Val4	435	360.94	
Val5	616	INVALID	Was not repeated
Val6	892	842.41	
Val7	218	OORL	Consistent
Val8	192	OORL	Consistent
Val9	279	263.4026	
Val10	470	149.1324	Outlier (same result after rerun)
Val11	833	OORH	Conistent
Val12	887	679.6859	
Val13	887	INVALID	Was not repeated
Val14	779	765.9457	
Val15	192	237.8725	

Val16	379	388.65	
Val17	748	873.28	
Val18	237	267.24	
Val19	604	787.84	
Val20	707	651.17	
Val21	428	467.12	
Val22	477	482.66	
Val23	474	444.14	
Val24	349	414.60	
Val25	549	517.28	
Val26	494	357.1135	
Val27	347	488.9352	
Val28	241	221.9306	
Val29	203	157.1844	
Val30	408	654.0567	

6 DILUTION LINEARITY

6.1 A pool of high analyte clinical samples was prepared and serially diluted with pool of low analyte clinical samples to generate a total of 9 points to test linearity. The nominal values of each level were reported by the predicate method (Siemens Immulite).

6.2 Acceptance criteria: 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 ± 20% of their nominal value, and when plotted, the R^2 value should be equal or greater than 0.95.

Overall, all but two levels recovered within 100 ± 20% of their CLIA value. Dilutions 5 and 7 recovered within 100 ± 25% of their CLIA value. When Theranos values are plotted against values determined by the predicate method, the fit equation is $y = 1.01x - 49.4$ with an R^2 value of 0.9.

Table 6: Dilution Linearity

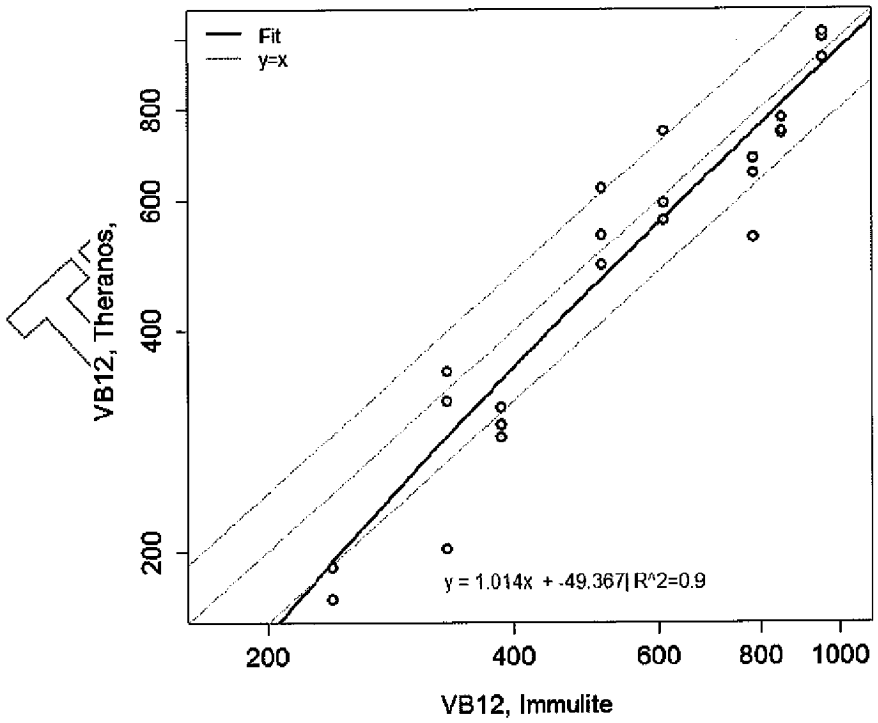
		RLU						Theranos Measured Concentration (pg/mL)						
Sample ID	CLIA Value (pg/mL)	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc1	Conc2	Conc3	Conc4	Conc5	Conc6	Result
High Patient Sample	951	2329	1640	2977	2365	2687	1048	907	OORH	741	804	669	OORH	987
		3829	3182	3544	3314	4127	3543	OORH	784	641	833	617	805	
		3582	4068	3031	3508	3932	4390	805	665	964	953	673	703	
Dilution 1	849	4335	4416	1147	4102	3884	4487	909	596	OORH	541	647	585	755
		3638	3566	3016	3936	976	3338	626	601	731	498	OORH	634	
		5063	4749	4860	4916	4791	4853	564	580	544	688	553	636	
Dilution 2	785	5515	4480	5131	5067	4648	5233	652	589	441	432	567	501	625
		3576	3371	4491	3712	3601	4524	635	624	499	525	550	482	
		6220	5948	5342	6272	5483	401	429	390	469	453	481	OORH	
Dilution 3	609	6429	6514	5238	6178	7138	5817	461	468	506	555	408	533	636
		4192	4543	4704	4259	3634	4019	583	451	527	645	686	691	
		6039	7305	8028	6045	5542	1261	485	431	354	566	491	OORH	
Dilution 4	512	10680	6383	7143	6183	6190	11529	313	475	388	554	451	289	551
		7096	3803	6184	5859	7189	6223	391	504	412	482	392	496	
		3816	3293	6082	6478	3229	4025	601	634	373	321	590	536	
Dilution 5	386	14618	10855	11579	11292	12727	12636	248	323	268	319	282	268	300
		12047	12925	11438	14009	13979	10559	275	265	250	223	240	336	
		18399	29819	29272	25808	26707	29262	127	175	169	168	240	166	
Dilution 6	331	11765	9471	10245	10563	13324	13899	237	363	287	266	330	252	292
		9045	12718	15448	8994	11041	10485	354	286	216	392	308	314	
		12372	15213	19365	21734	19345	18753	270	242	167	152	184	207	
Dilution 7	240	31185	69	29489	24739	33887	30922	126	OORH	125	156	123	127	182
		16034	10131	12312	18975	14311	11193	159	258	181	119	164	212	
		18704	19997	21601	19410	25962	24378	124	231	196	195	243	183	
Synthetic Serum	<150	43999	55075	59329	50438	45450	62030	OORL	OORL	OORL	OORL	OORL	OORL	OORL
		38728	40923	55802	47796	46934	43291	OORL	132	118	117	175	134	
		34781	36737	47119	36491	38841	46472	OORL	147	131	139	197	129	

Table 7: Dilution Linearity Summary

Sample ID	Nominal Value [pg/mL]	Theranos Result [pg/mL]	% Recovery
High Patient Sample	951	987	104%
Dilution 1	849	755	89%
Dilution 2	785	625	80%
Dilution 3	609	636	105%
Dilution 4	512	551	108%
*Dilution 5	386	300	78%
Dilution 6	331	292	88%
*Dilution 7	240	182	76%
Low Patient Sample	<150	OORL	

*Note: Dilution 5 and 7 each had 1 poor cartridge that brought the recovery down to slightly outside of the accepted range.

Figure 4: Dilution Linearity



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 193-982 pg/mL.

Samples were collected from 20 unique reference donors. These samples were confirmed via predicate method listed in section 1.2. 1 out of 20 patients had vitamin B12 levels outside of the reference range. Therefore 19 patient samples were tested using Theranos assay, one of which was outside of the reference range.

Overall, 95 % of the patients tested fell within the reference range, and is acceptable for verification of the reference range.

Table 8: Summary of normal patient samples for Reference Range

Sample ID	Reported Value [pg/mL]	Theranos Mean [pg/mL]
Patient 1	576	501
Patient 2	416	493

Patient 3	708	620
Patient 4	516	374
Patient 6	604	820
Patient 7	342	470
Patient 8	423	380
Patient 9	480	343
Patient 10	477	380
Patient 11	474	418
Patient 12	240	182
Patient 13	582	489
Patient 14	517	430
Patient 15	269	405
Patient 16	349	651
Patient 17	852	628
Patient 18	549	411
Patient 19	512	483

8 BLOOD COLLECTION DEVICE (BCD) COMPARISON

To compare EDTA plasma collected from Theranos blood collection device and venipuncture, 19 unique patients each donated 2 venous tubes of blood and 2 fingerstick samples. EDTA plasma was collected from venous and fingerstick blood collection and was tested on Edison readers.

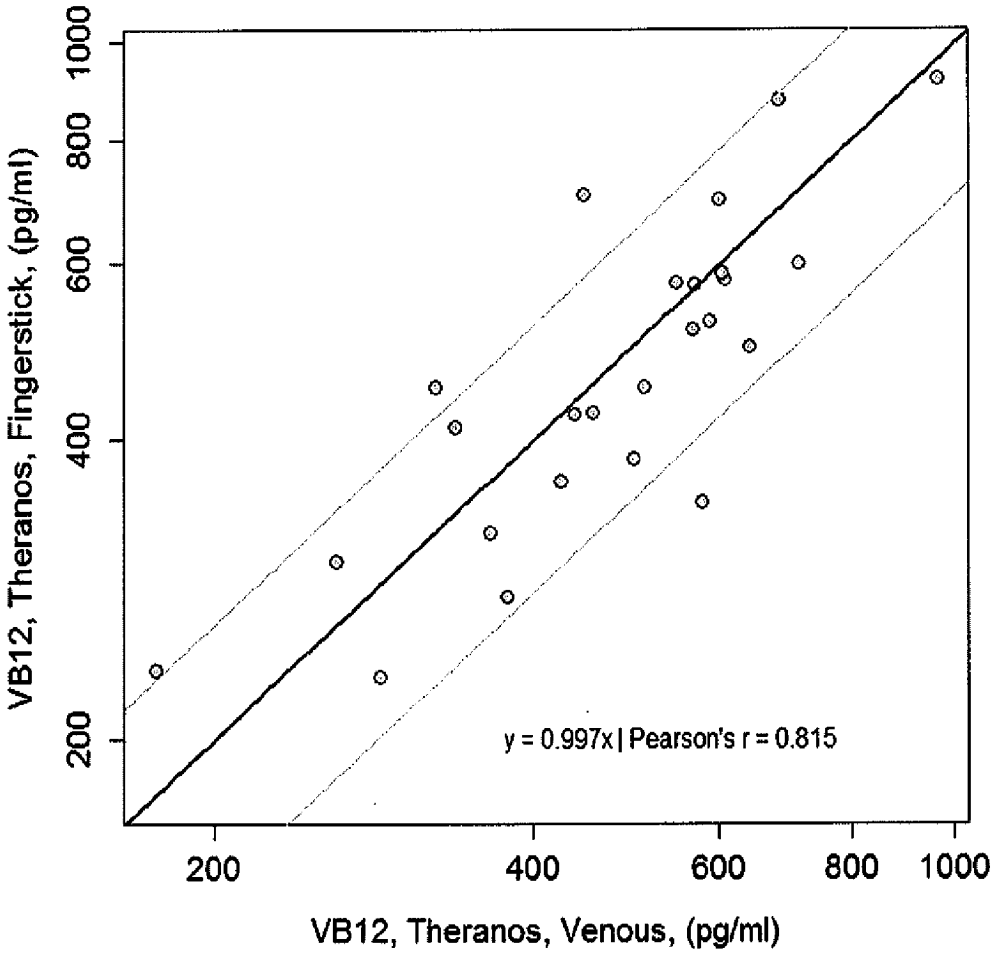
Results of fingerstick samples correlated with results of venous samples. The slope is 0.92, the R^2 of correlation is 0.77. The intercept of the fit line was forced to 0 to compensate for the narrow sample concentration range.

Table 9: Venous v. Fingerstick results

Sample	Venous	Fingerstick
Patient 1	not run	not run
Patient 2	963	919
Patient 3	456	426
Patient 4	568	572
Patient 5	588	525
Patient 6	608	579
Patient 7	424	363

Patient 8	567	515
Patient 9	438	424
Patient 10	509	452
Patient 11	546	575
Patient 12	287	231
Patient 13	712	601
Patient 14	579	346
Patient 15	641	495
Patient 16	603	587
Patient 17	599	696
Patient 18	338	411
Patient 19	498	382
Patient 20	261	302
Patient 21	403	183
Patient 22	323	452
Patient 23	683	877
Patient 24	447	704
Patient 25	809	1505
Patient 26	not run	not run
Patient 27	365	322
Patient 28	378	278
Patient 29	176	234
Patient 30	not run	not run

Figure 5: Comparison of fingerstick and venous blood samples


VB12, Theranos, Fingerstick, (pg/ml)
200
400
600
800
1000
y = 0.997x | Pearson's r = 0.815
200
400
600
800
1000
VB12, Theranos, Venous, (pg/ml)

9 ANALYTICAL SENSITIVITY

9.1 Verification of Limit of Blank

9.1.1 21 replicates of blank were measured and calculated from calibration curve.

9.1.2 Zero out of 21 replicates had quantifiable results.

9.2 Verification of Limit of Quantification

9.2.1 24 replicates of sample at LLOQ level were measured and calculated from calibration curve.

9.2.2 21 out of 24 replicates had quantifiable results.

9.3 Replicates of ½ LLOQ

9.3.1 The LLOQ sample was diluted 1:1 with blank and 21 replicates of the 1/2xLLOQ sample were tested.

9.3.2 2 out of 21 replicates had quantifiable results.

Table 10: Verification of blank

	RLU						Measured Concentration (pg/mL)						
Sample ID	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	result
Blank	66003	52979	68126	69283	71195	30040	OORL	OORL	103.30	OORL	120.20	163.71	OORL
Blank	28853	34887	36236	19855	24519	26518	OORL	OORL	OORL	113.87	OORL	OORL	OORL
Blank	38582	13769	42810	39411	36716	45303	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	44634	34491	40256	29045	32756	34409	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	79786	58691	69954	67238	60275	71080	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	74919	63019	32350	65226	73311	72570	OORL	OORL	113.84	OORL	OORL	OORL	OORL
Blank	25815	32938	23989	34699	29673	33900	OORL	OORL	108.34	OORL	OORL	OORL	OORL
Blank	48638	40003	41646	39822	44884	43730	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	22706	37099	27636	43058	35467	34887	111.48	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48934	47330	50648	52720	48453	37374	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	42379	48617	51483	42116	49537	51734	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	50133	50298	51513	39074	35335	51500	97.87	OORL	OORL	OORL	92.24	OORL	OORL
Blank	60246	47272	40190	43344	50702	58827	OORL	OORL	92.55	OORL	OORL	OORL	OORL

Blank	26973	51454	32035	39075	41992	43522	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	23807	43635	38774	44089	33351	30802	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	62602	64756	68704	67833	64711	58723	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	44913	31099	69181	75482	29647	48522	108.08	OORL	OORL	OORL	118.49	OORL	OORL
Blank	64772	67983	51877	85814	57739	53721	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	64922	38664	63995	76285	47163	59943	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	56109	83925	34635	13323	69272	58783	OORL	OORL	105.65	274.32	OORL	OORL	OORL
Blank	15120	48290	20812	59845	57607	56642	241.48	OORL	170.91	OORL	OORL	OORL	OORL

Table 11: Verification of LLOQ

	RLU						Measured Concentration (pg/mL)						
Sample ID	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	result
LLOQ	29458	28929	27768	34015	21383	24291	OORL	OORL	OORL	OORL	OORL	OORL	OORL
LLOQ	15324	18223	13086	16240	15876	11003	167	OORL	168	140	115	215	161
LLOQ	24447	24241	22612	40058	25982	31893	OORL	203	192	131	243	159	186
LLOQ	34978	40143	29610	36784	41930	33555	109	OORL	125	107	OORL	118	115
LLOQ	17215	20734	26162	19935	22804	18623	218	194	132	164	158	208	179
LLOQ	21162	18538	18946	21844	29389	33994	120	121	136	143	OORL	OORL	130
LLOQ	28956	24614	30923	28653	23849	23183	OORL	201	164	159	253	188	193
LLOQ	37939	31332	27945	33809	28046	36134	97	106	132	116	155	111	120
LLOQ	38049	36854	25848	33825	41737	26836	124	OORL	133	104	OORL	136	124
LLOQ	18964	15487	19186	17332	17653	18961	133	165	134	187	139	123	132
LLOQ	28451	26299	27043	22855	18920	27255	139	140	136	168	216	141	139
LLOQ	20233	20553	16599	16814	16398	22146	196	195	188	190	212	173	192
LLOQ	10476	12460	12415	10386	15660	14066	245	162	179	212	123	169	182
LLOQ	26676	16084	9610	22091	17581	11466	OORL	265	297	182	291	283	284
LLOQ	17851	20254	11684	20742	11965	13113	140	OORL	204	157	215	180	179
LLOQ	27672	24327	26671	22280	26589	27953	OORL	OORL	OORL	OORL	OORL	OORL	OORL
LLOQ	6647	21650	26728	18963	25276	20681	448	177	137	199	171	177	172
LLOQ	33759	29202	48706	31218	35886	39327	OORL	177	128	152	206	142	161
LLOQ	34569	42946	31483	35802	25818	34143	134	OORL	114	99	139	OORL	121
LLOQ	24726	19949	22826	20430	23714	24495	OORL	OORL	114	160	OORL	OORL	OORL

Table 12: ½ LLOQ Replicates

	RLU						Measured Concentration (pg/mL)						
Sample ID	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	result
1/2 LLOQ	23234	27163	33947	32761	32386	32064	104.52	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	50006	49932	30624	52345	43240	42549	98.10	OORL	116.36	OORL	OORL	OORL	OORL
1/2 LLOQ	43231	29176	36972	37912	38845	39149	OORL	120.13	97.90	204.43	97.66	103.67	100.91
1/2 LLOQ	44205	51459	49385	51053	59536	51360	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	47959	33879	33842	37682	34209	43866	101.95	OORL	187.15	94.27	97.12	OORL	100.12
1/2 LLOQ	32400	30794	32133	33416	31123	31540	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	32873	28977	33745	29105	24134	27502	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	40372	36918	37587	41322	37226	42400	118.31	OORL	98.06	OORL	OORL	OORL	OORL
1/2 LLOQ	39476	65733	59941	15886	66793	38719	OORL	OORL	OORL	233.80	OORL	104.65	OORL
1/2 LLOQ	40856	32273	35181	9974	32135	34069	OORL	OORL	OORL	219.93	OORL	OORL	OORL
1/2 LLOQ	33953	32659	29638	32284	27386	27898	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	31793	32650	28074	27107	27264	32569	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	78725	101176	96011	91661	73426	91902	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	35566	27612	27258	27899	32108	28609	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	26864	30812	32347	27399	27764	32598	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	43509	39680	43557	46381	40242	45049	111.09	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	47950	46421	34320	41612	46336	42465	101.97	OORL	105.91	OORL	OORL	OORL	OORL
1/2 LLOQ	11399	36448	36051	39538	40874	41093	284.85	OORL	101.61	OORL	OORL	OORL	OORL
1/2 LLOQ	60149	36011	38844	61295	54148	59941	OORL	OORL	95.33	OORL	OORL	OORL	OORL
1/2 LLOQ	41058	39817	35960	42158	44737	39784	116.67	OORL	101.83	OORL	OORL	OORL	OORL

Table 13: Analytical Sensitivity Summary

Statistic	Percentage
% of blanks that are OORL	100
% of LLOQ that are calculable	88
% of 1/2 LLOQ that are OORL	90

10 ANTICOAGULANT COMPARISON

To test the effect of anticoagulants, 19 unique patients donated a total of 3 samples, one EDTA, one Li Heparin, and one Serum. The matched samples were tested in triplicate on Theranos systems and the results were compared across three matrices. There was no significant difference between serum, EDTA and heparin plasma. EDTA plasma, Li-heparin plasma and serum are suitable for the Theranos vitamin B12 assay. When plotted against each other, the fit line was forced to an intercept of 0 to compensate for the narrow sample concentration range.

Table 14: EDTA Plasma

Sample ID	CLIA conc. pg/mL	RLU						Measured Concentration (pg/mL)						
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Tip 1	Tip 2	Tip 3	Tip 4	Tip 5	Tip 6	Mean
Patient 1	576	7072	6148	5869	7602	6011	6549	392	383	431	382	448	478	501
Patient 2	416	6064	5664	6338	5604	4753	4944	404	444	358	365	463	446	493
Patient 3	708	3907	6589	5053	5068	4934	6616	619	370	493	550	524	475	620
Patient 4	516	8045	9694	9196	9364	11066	8384	359	307	297	317	286	399	374
Patient 5	604	4455	2539	3020	3552	2819	3046	528	758	731	547	647	689	820
Patient 6	342	7489	6660	6806	6458	8036	7984	433	451	363	358	417	358	470
Patient 7	423	7856	10123	6275	9888	6431	4927	320	258	362	222	382	447	380
Patient 8	480	9541	8354	11048	10300	10781	10380	320	330	257	291	292	341	343
Patient 9	477	9214	8845	10621	11741	9856	9494	349	376	286	308	331	342	380
Patient 10	474	9665	7822	7635	7108	8636	7763	308	408	338	336	403	365	418
Patient 11	240	11499	14658	12335	13483	12190	14974	224	OORL	180	167	218	159	182
Patient 12	582	5810	5201	4755	4947	6956	6832	420	471	472	407	362	334	489
Patient 13	517	9709	7397	8005	6181	7556	9168	316	350	332	459	378	374	430
Patient 14	269	8412	6347	9012	9060	9528	11363	371	465	307	290	385	284	405
Patient 15	349	3843	4221	4627	3922	6208	3233	598	540	485	500	391	653	651
Patient 16	852	4249	4335	4543	4265	3954	4243	549	530	493	464	518	511	628
Patient 17	549	8181	6786	8163	5787	7299	10504	355	365	326	488	388	338	411
Patient 18	512	6154	6912	8969	6924	2482	7410	562	440	308	341	OORH	378	483

Patient 19	608	7631	9685	8379	8184	7494	8378	403	352	343	428	395	382	452

Table 15: **Li Heparin Plasma**

Sample ID	CLIA conc. pg/mL	RLU						Measured Concentration (pg/mL)						
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Tip 1	Tip 2	Tip 3	Tip 4	Tip 5	Tip 6	Mean
Patient 1	576	4393	3124	1038	3671	3790	3229	534	656	OORH	530	532	653	657
Patient 2	416	1899	9289	6877	9011	7290	7999	OORH	368	360	291	438	358	422
Patient 3	708	5794	5913	5661	6287	5911	6315	502	502	472	546	467	494	609
Patient 4	516	6449	6329	5390	6531	6016	5681	383	440	419	319	399	394	475
Patient 5	604	6003	3929	3606	2412	4546	107	408	565	615	788	475	OORH	710
Patient 6	342	10504	12234	9945	7654	9304	10261	276	312	291	320	389	303	336
Patient 7	423	9119	7184	7771	7492	10130	10620	352	436	363	464	326	310	440
Patient 8	480	9904	9290	9040	10059	2247	10426	331	363	323	354	OORH	315	387
Patient 9	477	8228	6949	6298	7220	8571	7239	382	439	381	332	405	384	458
Patient 10	474	5149	9335	5045	5441	7920	6047	494	313	494	516	365	507	616
Patient 11	240	18285	15671	13974	8350	12384	16598	188	OORL	156	257	213	142	122
Patient 12	582	9261	3931	10448	7291	5912	9571	326	493	268	396	454	362	451
Patient 13	517	3943	5225	4492	5480	4793	4983	585	469	499	372	460	443	568
Patient 14	269	8703	10067	8400	6915	8738	10756	355	350	320	342	401	294	396
Patient 15	349	1757	7918	8390	8089	8203	8529	OORH	339	319	361	356	394	410
Patient 16	852	5643	4784	4352	4784	6201	4960	632	562	508	455	477	527	650
Patient 17	549	6575	7301	7470	8154	9080	10296	412	352	351	358	330	343	401
Patient 18	512	8952	7572	7116	8440	8620	7715	357	420	390	416	361	411	464
Patient 19	608	8727	10700	8537	11307	9140	10486	340	293	315	268	329	338	355

Table 16: Serum

Sample ID	CLIA conc. pg/mL	RLU						Measured Concentration (pg/mL)						
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Tip 1	Tip 2	Tip 3	Tip 4	Tip 5	Tip 6	Mean
Patient 1	576	5948	5049	2856	5799	3696	6598	589	541	1028	387	675	412	759
Patient 2	416	4649	5993	6288	5906	10944	6361	610	497	431	579	310	491	594
Patient 3	708	5132	3848	4894	3600	5549	7461	495	500	508	754	477	435	589
Patient 4	516	10105	16193	13041	8810	10368	17070	291	264	253	295	370	224	311
Patient 5	604	4116	3010	3185	3752	4821	3312	564	673	694	520	459	638	739
Patient 6	342	14396	12236	11534	10004	12355	15640	251	295	269	356	287	224	308

Patient 7	423	12403	13808	7293	9425	12351	11845	270	256	358	315	264	308	329
Patient 8	480	24182	19805	6194	24370	14799	34066	OOR L	232	386	173	315	153	269
Patient 9	477	6184	7200	5670	6600	4312	6730	397	370	399	316	491	339	454
Patient 10	474	6665	4907	5599	8990	4787	7857	447	576	477	392	554	405	578
Patient 11	240	17620	25942	18260	18249	18967	22926	214	142	190	206	216	163	182
Patient 12	582	10630	11112	12539	10005	2677	22537	272	330	258	274	OORH	191	291
Patient 13	517	7317	7867	9526	4603	8534	11178	416	409	311	732	363	297	418
Patient 14	269	9368	7161	13495	4662	12347	13717	345	437	239	723	287	250	352
Patient 15	349	9102	5946	5121	6943	7467	6410	330	390	487	414	381	486	495
Patient 16	852	7315	5513	6299	5495	6394	4009	446	510	381	403	469	670	532
Patient 17	549	4304	4403	4852	3940	4193	4287	543	525	463	498	500	506	621
Patient 18	512	8713	6155	6101	7700	6319	7198	364	488	442	452	445	438	536
Patient 19	608	6546	4558	5854	6386	5939	7194	414	450	432	446	452	447	542

Table 17: Summary of EDTA v. Li Heparin v. Serum

Sample ID	Reported Value [pg/mL]	Theranos		
		Li-hep	serum	EDTA
Patient 1	576	657	759	501
Patient 2	416	422	594	493
Patient 3	708	609	589	620
Patient 4	516	475	311	374
Patient 5	604	710	739	820
Patient 6	342	336	308	470
Patient 7	423	440	329	380
Patient 8	480	387	269	343
Patient 9	477	458	454	380
Patient 10	474	616	578	418
Patient 11	240	122	181	182
Patient 12	582	451	291	489
Patient 13	517	568	418	430
Patient 14	269	396	352	405
Patient 15	349	410	495	651

Patient 16	852	650	532	628
Patient 17	549	401	621	411
Patient 18	512	464	536	483
Patient 19	608	355	542	452
Patient 1	576	657	750	501
IHP1	300			
IHP2	270		423	303
IHP3	397		185	160
IHP4	573			
IHP5	306		276	216
IHP6	632		611	510
IHP7	759		865	
IHP8	526		597	409
IHP9	886		730	1165
IHP10	340		301	327
IHP11	687		745	521
IHP12	406		479	445
IHP13	560		651	392
IHP14	584		329	368
IHP15	312		226	336
IHP16	422		517	327
IHP17	819		621	615
IHP18	363		308	
IHP19	971		1489	1176
IHP20	642		555	663

Figure 6: EDTA Plasma Vs Serum. The 95% confidence interval on the slope is [1.005, 1.040]. The grey lines are unity and 30% error on either side of unity.

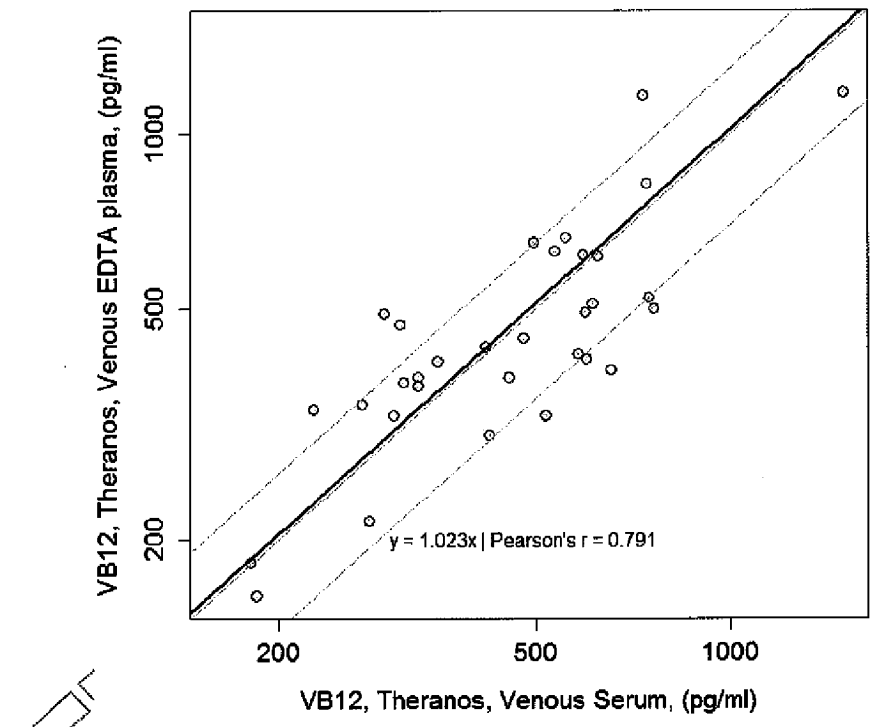
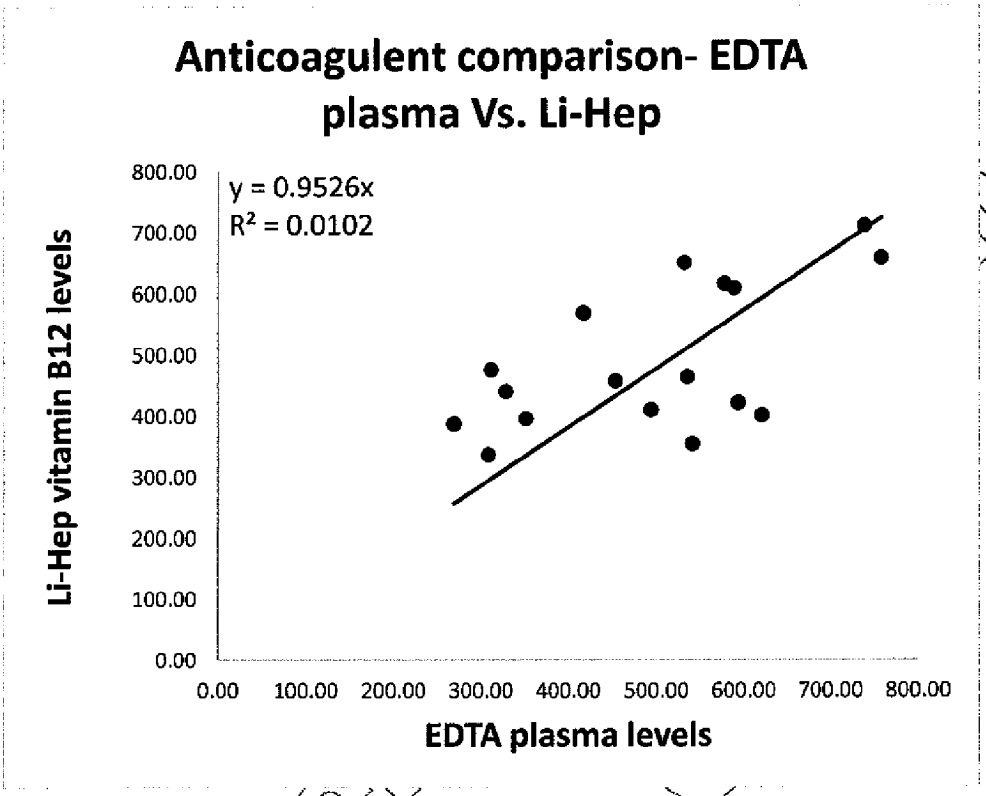


Figure 7: EDTA Plasma Vs Li-Hep



11 INTERFERENCE

Matrix interference was evaluated by preparing hemolyzed, icteric and lipemic matrices and spiking three concentration levels of the analyte into each prepared matrix respectively.

The spiked serum samples were measured in triplicate on the Theranos System as well as on the predicate system and % recovery was calculated.

Vitamin B12-spiked icteric samples containing 10 mg/dL of bilirubin and vitamin B12-spiked hemolyzed samples containing 50 mg/dL of hemoglobin showed acceptable recoveries when compared with the predicate method, which indicates a lack of interference. Higher levels of bilirubin (20 mg/dL) and hemoglobin (100 mg/dL) showed interference. The Theranos assay shows interference when used to evaluate vitamin B12-spiked lipemic samples with triglyceride concentrations of 250 mg/dL, 1000 mg/dL, and 4000 mg/dL. Experiments are ongoing to determine the highest concentration of triglycerides which does not interfere with the assay.

Hence, results of the Theranos assay from grossly lipemic samples, as well as samples containing hemoglobin above 50 mg/dL and bilirubin above 10 mg/dL, should be interpreted with caution.

Table 18: Results of Hemolyzed samples using 50 mg/dL Hemoglobin

Spiked VB12 [pg/mL]	All Tips						Concentration							
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Mean	% Recovery
696	4132	4338	4741	4232	3959	4412	562	530	474	467	518	493	686	99%
696	4059	4112	4423	3811	3879	4900	599	479	560	715	645	593		
696	3091	3304	3411	3469	4389	3220	978	790	810	964	600	977		
423	5632	5856	4966	4907	5201	4746	431	434	453	410	438	462	707	167%
423	4065	3643	4173	3442	3521	2687	598	520	593	786	708	985		
423	5871	6378	6229	5902	7306	5813	496	475	435	579	402	534		
220	9712	8985	11579	12372	11243	11320	263	300	193	181	241	210	257	117%
220	13453	13425	14002	12394	18348	14680	256	260	214	248	193	258		
220	16924	15062	16649	16743	17971	14068	221	249	204	223	224	244		
<150	28873	30091	32552	34828	35671	31711	OORL	OORL	OORL	OORL	OORL	OORL	OORL	-
<150	74359	68773	48724	51762	52894	50707	OORL	OORL	OORL	OORL	OORL	OORL		
<150	55114	53383	61787	64161	59885	37183	OORL	OORL	OORL	OORL	OORL	141		

Table 19: Results of Hemolyzed samples using 100 mg/dL Hemoglobin

Spiked VB12 [pg/mL]	All Tips						Concentration							
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Mean	% Recovery
721	2539	3256	2569	2876	2935	3092	844	638	857	665	629	680	908	126%
721	3779	3493	3354	3426	3575	4814	756	753	829	976	757	641		
721	2586	3358	2985	3147	3100	3436	944	553	858	855	809	787		
476	4601	4834	4552	4693	4990	4288	540	435	544	590	519	657	720	151%
476	4258	2966	3015	2964	3135	2868	548	679	732	646	601	728		
476	4977	5833	5340	6155	1765	6063	573	507	498	557	OORH	513		
241	13190	13947	14021	13886	11649	13646	268	266	233	264	298	251	309	128%
241	14223	11643	13281	12518	11962	13442	247	280	223	246	270	278		
241	9116	10493	11245	10846	10380	3042	352	331	274	331	321	OORH		
<150	44428	42114	34852	45389	37689	36520	OORL	OORL	OORL	OORL	OORL	OORL	OORL	
<150	50124	49569	49036	58894	63008	57257	98	OORL	OORL	OORL	OORL	OORL		
<150	32598	32456	35736	44297	30891	39859	OORL	OORL	OORL	OORL	OORL	OORL		

Table 20: Results of icteric samples using 10 mg/dL bilirubin

Spiked VB12 [pg/mL]	All Tips						Concentration							
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Mean	% Recovery
614	1133	3554	4530	3037	3765	4005	OORH	603	495	632	534	538	596	97%
614	6576	6093	5935	7099	6321	7637	412	385	427	406	431	428		
614	6250	5692	4939	5180	6256	6595	471	516	535	655	448	474		
432	6003	5280	5527	5201	5122	6365	408	466	409	389	442	356	427	99%
432	7787	7464	8146	7923	7652	10246	367	348	327	368	374	344		
432	10191	9689	10188	9310	11706	9513	324	352	295	380	297	342		
239	19437	18545	16918	17891	20682	20360	198	207	202	210	202	179	168	70%
239	12933	14302	12422	10556	12282	14526	199	OORL	179	209	215	164		
239	25099	34237	24614	23439	25830	23524	169	OORL	139	142	139	162		
<150	60056	57140	57376	57447	67154	57951	OORL	OORL	OORL	OORL	OORL	OORL	OORL	
<150	60454	62121	65163	64306	55304	54015	OORL	OORL	OORL	OORL	OORL	OORL		
<150	39190	41803	42966	51330	42762	39460	OORL	OORL	OORL	OORL	OORL	OORL		

Table 21: Results of Icteric samples using 20 mg/dL Bilirubin

Spiked VB12 [pg/mL]	All Tips						Concentration							
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Mean	% Recovery
713	6578	5816	5840	5306	5840	4939	376.03	435.64	387.71	382.48	406.72	446.24	319	45%
713	704	329	356	265	525	233	OORH	OORH	OORH	OORH	OORH	OORH		
713	23788	24013	13739	24296	15378	32295	175.50	166.84	217.15	137.86	222.82	99.70		
459	8105	6388	8634	8778	8170	5920	311.45	406.88	263.39	246.35	322.61	379.82	410	89%
459	9798	7026	6198	9561	6892	12173	333.68	443.30	436.52	370.97	418.41	276.23		
459	7003	9604	8873	7204	10345	13994	430.30	353.84	327.78	481.00	321.29	245.46		
284	16803	7603	17019	16838	15912	13101	151.35	353.48	122.71	134.70	113.50	181.68	122	43%
284	23984	28167	28786	17350	22192	27718	174.51	128.31	122.35	184.65	162.34	129.71		
284	10059	50211	86543	80540	71438	46952	327.26	OORL	OORL	OORL	OORL	OORL		
<150	45121	45282	40947	41306	40350	41148	OORL	OORL	OORL	OORL	OORL	OORL	OORL	
<150	71521	58722	77883	41941	46922	68765	OORL	OORL	OORL	OORL	OORL	OORL		
<150	80663	50204	61966	55203	65028	51802	OORL	OORL	OORL	OORL	OORL	OORL		

Table 22: Results of Lipemic samples using 250 mg/dL Triglycerides

Spiked VB12 [pg/mL]	All Tips						Concentration							
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Mean	% Recovery
698	3793	3472	3748	3820	3371	3570	636	539	662	713	740	762	837	120%
698	3923	4026	3600	3994	3886	3532	617	485	691	684	644	769		
698	3952	3419	3414	3520	9	3901	720	767	809	950	INVALID	794		
408	1237	620	506	710	612	557	OORH	OORH	OORH	OORH	OORH	OORH	738	181%
408	4226	4962	4494	4491	4191	4759	579	429	551	614	602	606		
408	3570	3969	3935	4585	4405	4268	808	678	678	735	598	723		
219	2163	8306	8264	7409	7204	8724	964	326	275	286	354	268	306	140%
219	12064	12169	12704	12141	12630	11272	275	274	231	252	259	320		
219	14681	14056	14432	13451	14290	14765	247	264	228	272	262	235		
<150	32106	31158	38398	36119	26151	40631	OORL	OORL	OORL	OORL	OORL	OORL	OORL	
<150	5730	13238	11084	9435	10537	9061	456	262	256	315	297	377		
<150	49211	56794	50329	49034	9	48257	OORL	OORL	OORL	OORL	INVALID	OORL		

12 CROSS REACTIVITY

Vitamin B12 assay was tested for cross reactivity from high levels of dicyanocobinamide due to its structural similarity to vitamin B12. Dicyanocobinamide was spiked into synthetic serum substitute (with no Vitamin B12 present) at 1.25 µg/mL and 50 µg/mL and evaluated using the Theranos assay.

Despite the fact cross reactivity with Dicyanocobinamide was observed at 50 ug/mL (Level reported by the SIEMENS Immulitc 2000 VB12 test), no such cross reactivity was observed at 1.25 ug/mL. A level of 1.25 ug/mL of Dicyanocobinamide is over 1000 times higher than the physiological levels of cobinamides that occur in the human body.

Table 23: Cross-reactivity study

[dicyanocobinamide] µg/mL	Signal, RLU		
	Mean RLU	CV %	Mean Conc pg/mL
1.25	47190	30%	OORL
50	3949	23%	808

13 ANALYTE STABILITY

The stability of analyte was tested at 4C and room temperature to mimic the conditions that clinical samples which may be stored and handled. Samples were transferred from -80C to either room temperature or 4C at the 0 hour time point, and stored at these temperatures for the remainder of testing.

Overall the analyte stability data indicates that the samples are stable up to 72 hours when stored at 4C and up to 24 hours when stored at room temperature.

Table 24: Analyte Stability Summary: 4C

4C										
Level (pg/mL)	RLU					Calculated Concentration (pg/mL)				
	0hr	4hr	12hr	24hr	72hr	0hr	4hr	12hr	24hr	72hr
746	4029	3436	3600	ND	2570	886	783	861	ND	921
473	6204	4976	4417	4484	3877	641	683	658	704	768
212	10626	18773	17824	11768	14206	242	187	213	204	232

Table 25: Analyte Stability Summary: RT

RT										
Level (pg/mL)	RLU					Calculated Concentration (pg/mL)				
	0hr	4hr	12hr	24hr	72hr	0hr	4hr	12hr	24hr	72hr
746	3529	3764	2715	4389	3829	891	997	965	710	727
473	6204	6357	4737	4882	5626	641	522	656	681	619
212	12386	13293	15701	10174	21237	202	152	240	256	165

Figure 8: Analyte Stability at 4C

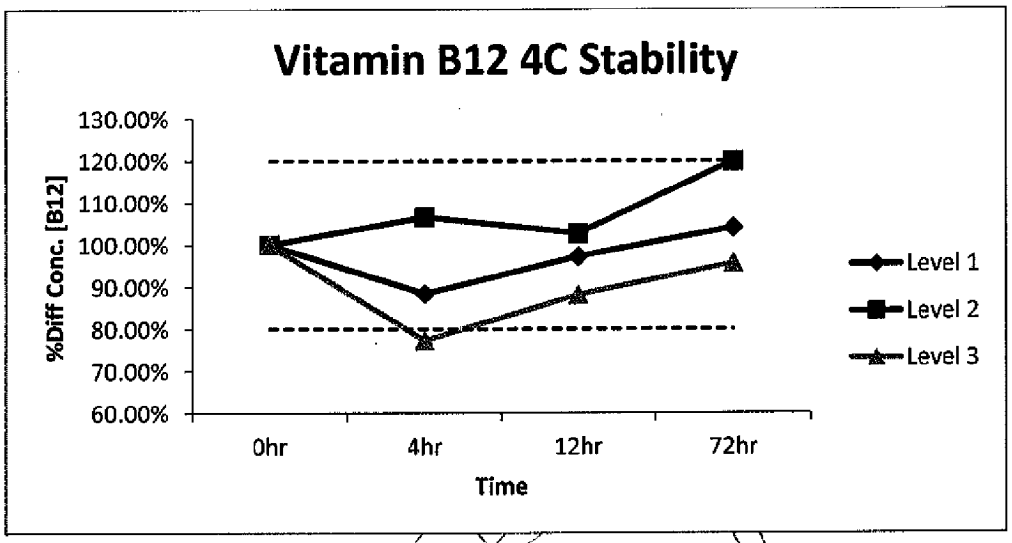
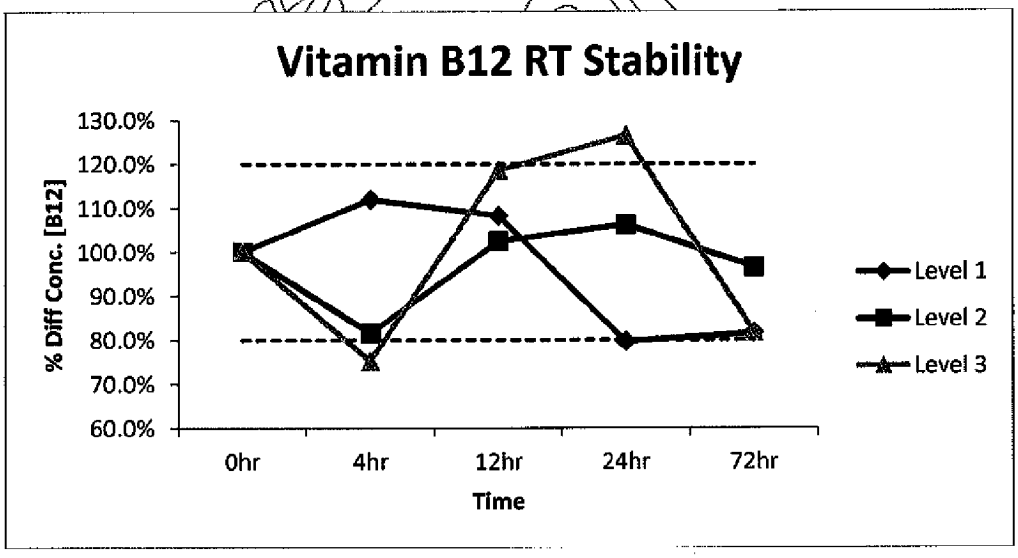


Figure 9: Analyte Stability at Room Temperature



14 Reagent Stability in Capsys Cartridges

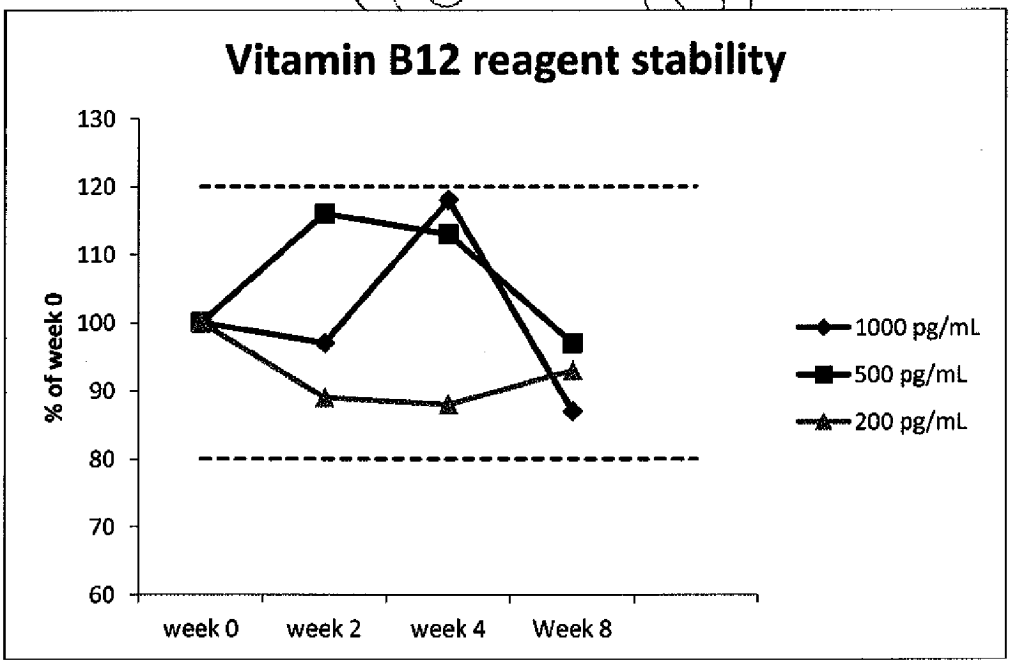
Reagent stability is conducted with Capsys built cartridges. Cartridges with all reagents filled by Capsys are stored at 4C and the monitoring of assay performance is on-going.

Bulk reagent stability using manually filled cartridges is also ongoing. Here we show data for up to 4 week of reagent stability stored at 4C using three different calibrator concentrations. Results are shown as a percent of the week 0 measurement.

Table 26: Bulk reagent Stability Summary

Level (pg/mL)	RLU				Calculated Concentration (pg/mL)			
	Week 0	Week 2	Week 4	Week 8	Week 0	Week 2	Week 4	Week 8
1000	4299	3848	3511	4478	1296	1259	1535	1127
500	7869	6533	7447	8003	442	514	499	430
200	21213	23837	23754	22772	197	175	174	184
0	60551	67937	58675	66752	-	-	-	-

Figure 10: Bulk reagent stability



15 CTN sample stability

15.1 To test the sample stability in blood collection devices (BCD), fingerstick samples from each donors were collected in six blood collection devices (BCD) at the same time.

15.2 Samples from 19 donors were tested in two arms of the study.

15.3 Study A involved collection of the donor samples in the BCD, followed by storage at 4C until the respective time point. Samples from this study were spun only at the particular time point just before the runs. This study most accurately represents what will occur clinically.

15.4 Study B involved spinning of the samples immediately after collection, followed by storage at 4C for the respective time points.

15.5 For both arms of the studies, Study A and Study B, the samples were tested at 0, 8, 24 and 48 hours for the sample stability in CTN.

15.6 Upon completion of this study, the samples show to be stable through 48 hours.

15.7 It is recommended for the Vitamin B12 assay that the samples not be spun until the time of testing modeling Study A.

Study A:

Table 27: CTN sample stability Study A

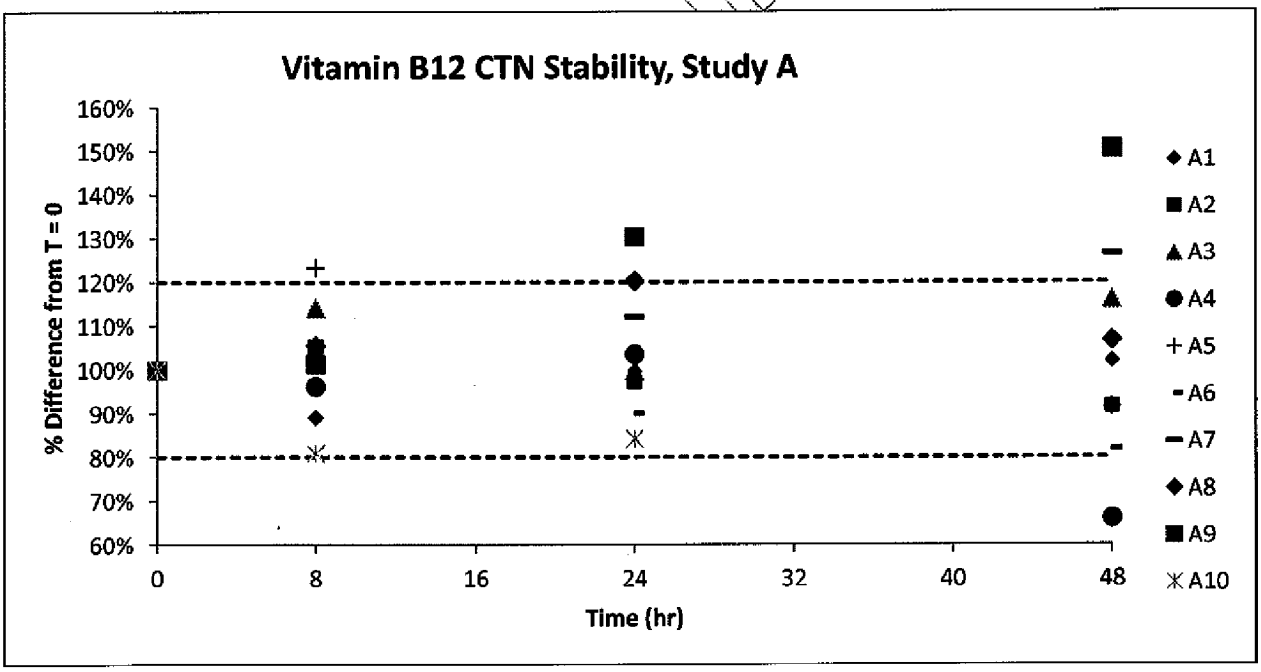
	Mean Conc. (pg/mL) per Patient									
Summary	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
0	454.2	249.4	383.4	586.6	201.3	563.6	234.2	440.9	627.4	264.7
8	404.6	262.9	503.0	564.5	248.4	569.7	240.3	465.5	635.7	214.2
24	452.2	242.5	382.5	607.3	-	507.4	262.4	530.4	818.1	222.9
48	463.2	228.5	444.7	387.4	184.3	461.8	296.2	470.5	944.1	-

Table 34: CTN sample stability Study A (% Concentration with respect to T = 0)

% Difference from T = 0 per Patient										
Summary	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
0	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
8	89%	105%	114%	96%	123%	101%	103%	106%	101%	80.93%
24	100%	97%	100%	104%	-	90%	112%	120%	130%	84.22%
48	102%	92%	116%	66%	92%	82%	126%	107%	150%	-

*Note: Patients A4 and A6 appeared to be hemolyzed at the 48 Hour Time Point

Figure 11: CTN sample stability Study A



Study B:

Table 35: CTN sample stability Study B

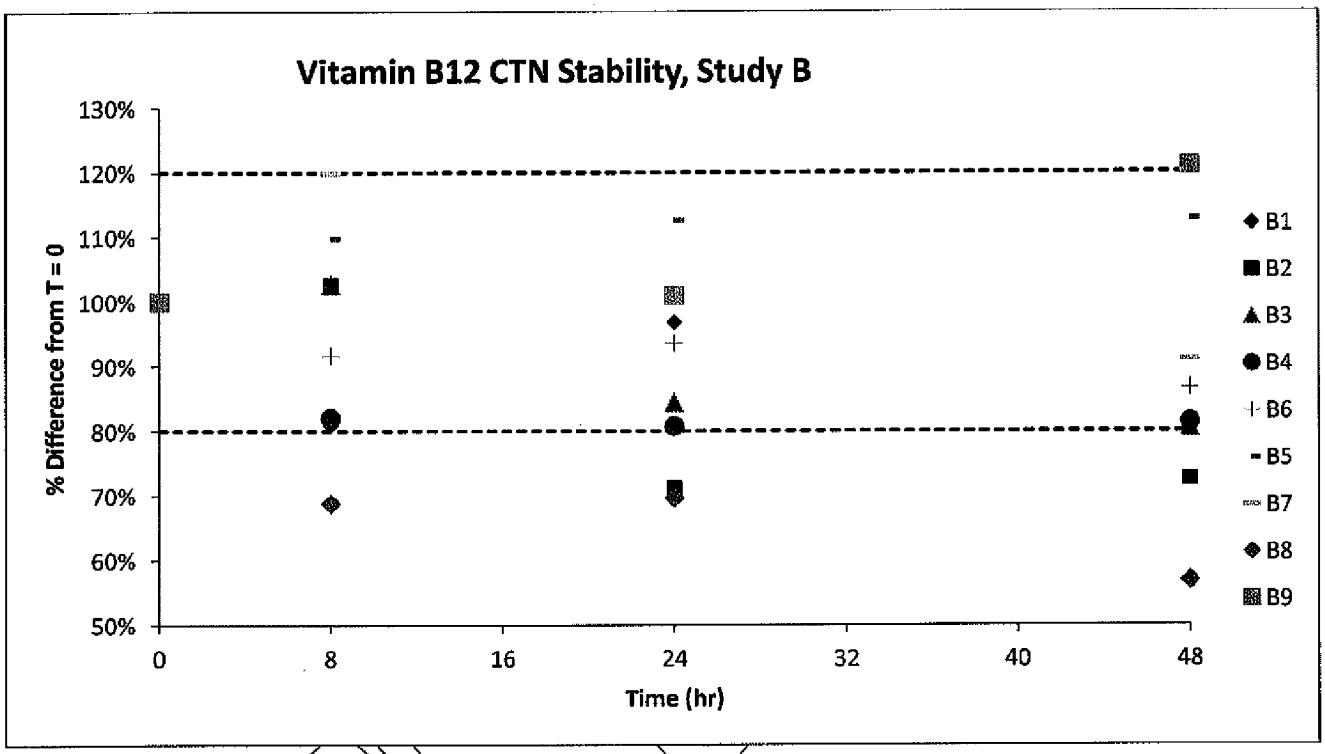
Mean Conc. (pg/mL) per Patient									
Summary	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	454.2	249.4	383.4	749.4	207.5	524.1	415.2	763.8	856.4
8	404.6	262.9	503.0	614.8	227.9	480.6	498.0	526.0	-
24	452.2	242.5	382.5	604.9	233.5	490.4	416.8	532.0	864.3
48	463.2	228.5	444.7	609.6	234.0	453.9	377.9	434.4	1036.1

Table 36: CTN sample stability Study B (% Concentration with respect to T = 0)

% Difference from T = 0 per Patient									
Summary	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	100%	100%	100%	100%	100%	100%	100%	100%	100%
8	89%	105%	114%	82%	110%	92%	120%	69%	-
24	100%	97%	100%	81%	113%	94%	100%	70%	101%
48	102%	92%	116%	81%	113%	87%	91%	57%	121%

***Note: Samples B1, B2, and B3 were hemolyzed from T = 0, and B6 was severely lipemic.**

Figure 18: CTN sample stability Study B



16 REFERENCES

16.1 Code of Federal Regulations, Title 42, Chapter IV, Subchapter G, Part 493, Subpart K, Sections 493.1217, 493.1253, and 493.1255.

16.2 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.

16.3 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.

16.4 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.

16.5 StatisPro (version 1.13.00). Clinical and Laboratory and Standards Institute, Wayne, PA. 07/14/2011.

16.6 Dexter-Immunoassay (version1.0), Theranos, Inc., 2009.

16.7 EP10-A3, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition, 2006, Clinical and Laboratory Standards Institute, Wayne, PA.

16.8 EP15-A2, User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. 2005, Clinical and Laboratory Standards Institute, Wayne, PA.

16.9 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.

16.10 EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

16.11 EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003, Clinical and Laboratory Standards Institute, Wayne, PA.

16.12 EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.