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Prolactin ELISA Assay Validation Report on Edison 3.5 Theranos System

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Table of Content

List of Tables

List of Figures

1 ASSAY BACKGROUND

Human Prolactin is a polypeptide hormone of the anterior pituitary with a molecular mass of about 22.8K Daltons. It plays an essential role in the secretion of milk and has the ability to suppress gonadal function. Women are reported to have slightly higher mean levels than men, with a slight rise at puberty (apparently estrogen related) and a corresponding fall at menopause. Prolactin stimulates the mammary glands to produce milk (lactation) and increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands of the breasts and prepare for the production of milk. Milk production normally starts when the levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. As a reference range for circulating prolactin, the literature suggests concentrations up to approximately 20ng/mL. Values are distinctly elevated at birth but decline to adult levels in less than three months. Women taking oral contraceptives or under estrogen treatment may have prolactin levels higher than normal.

In assessing the significance of moderate elevations, it is important to keep in mind that prolactin is a stress related hormone and it is directly proportional to stress. There is a sleep related diurnal variation as well (prolactin levels increase during sleep and reach their lowest a few hours after waking) and day-to-day fluctuations with CVs as high as 30% have been encountered.

Determination of prolactin has become an important tool in the investigation of amenorrhea, galactorrhea and hypothalamic-pituitary disorders.

Normal levels of Prolactin are summarized in Table 1 (Reference: IMMULITE assay)

Table 1: Reference Ranges

Group	N	Median (ng/mL)	Range
Adult male	19	6.2	2.5-17
Adult Females	155	9.4	1.9-25
Pediatric Patients			
Females : Cord	28	380	200-675
Females : 0.1-0.5yr	28	15	1-140
Females : 0.6-9yr	55	11	2-43
Males : Cord	27	295	150-565
Males : 0.1-0.5yr	36	19	4-65
Males : 0.6-9yr	55	8	0.6-29
Combined: Cord	55	340	160-665
Combined: 0.1-0.5yr	64	117	2-125
Combines: 0.6-9yr	110	9	1-40

1.1 Theranos System Specification

The Theranos Prolactin assay has a reportable range of 1.28-639.90 ng/mL. The Theranos Prolactin standards are calibrated against the World Health Organization's First International Standard for Prolactin, Human (Code 84/500). EDTA, Li-heparin plasma and Serum are acceptable sample types for the Theranos Prolactin assay. Samples can be processed up to 72h if stored at 4 degrees or room temperature.

Table 2 : Theranos System Specification table

Assay	Theranos Prolactin Assay
Reportable Range	1.28-639.90ng/mL
Gold Standard	WHO's First International Standard Prolactin, Human (Code84/500)
Accepted sample type	EDTA, Lithium Heparin plasma and Serum
Sample Stability	Up to 72 hours at 4 degree Celsius or Room Temperature

1.2 Reference Assay

The Siemens IMMULITE 2000 Prolactin assay was used as a predicate method. Lipemic samples are recommended to centrifuge before testing and hemolyzed sample results should be interpreted with caution. The assay reportable range is 0.5-150 ng/mL. The presence of bilirubin (200mg/L), hemoglobin (200mg/dL), and triglycerides (3000mg/dL) have no effect on the 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 The term "calibration verification," as used in CLIA, includes:

3.1.2.1 Confirming that a calibration meets the method manufacturer's specifications

3.1.2.2 Verifying that the calibration is suitable for the entire measuring interval (or "reportable range," which is the CLIA term)

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

3.1.3.1 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.

3.1.3.2 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 Calibration 1 (Edison 3.5, 20uL)

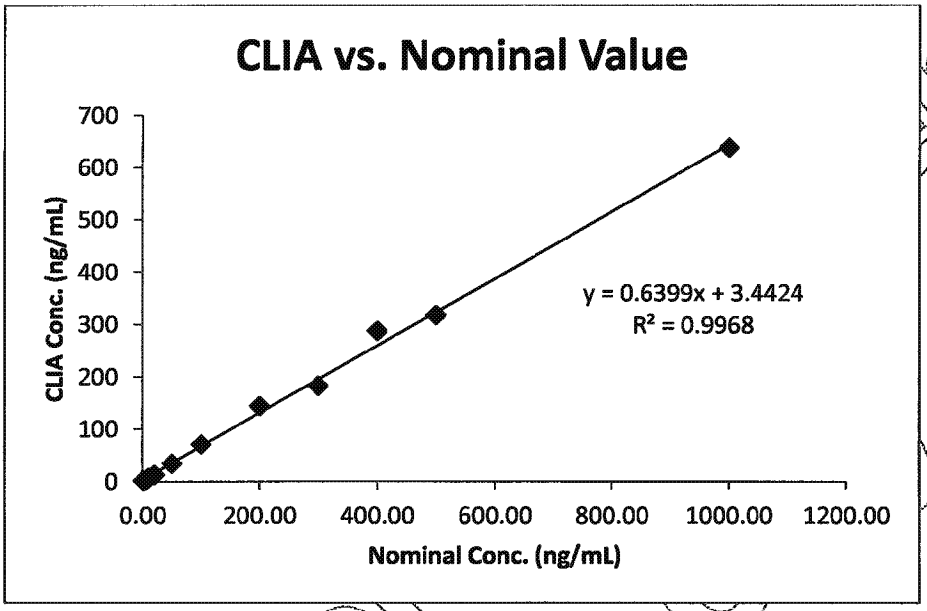
Of the 12 levels, all 11 (except for 0ng/ml) had a back calculated mean value within 100 ± 20% (100 ± 25% at LLOQ and ULOQ standards) of their nominal values.
The CLIA values are obtained from the CLIA lab, a graph is plotted (Nominal vs CLIA values) and the Reassigned is calculated from the slope.

Reassigned Value (ng/mL) = Nominal Value * 0.6399

Table 3 : Prolactin Calibrators

Calibrator #	Prolactin (ng/mL) Nominal Values	CLIA Values (ng/mL)	Re-assigned Conc. Ng/mL
1	1000.00	637	639.90
2	500.00	317	319.95
3	400.00	287	255.96
4	300.00	182	191.97
5	200.00	144	127.98
6	100.00	70.4	63.99
7	50.00	33.9	32.00
8	20.00	12.3	12.80
9	10.00	5.95	6.40
10	5.00	2.6	3.20
11	2.00	1.25	1.28
12	0.00	< 0.50	0

Figure 1 : CLIA vs Nominal Values (ng/mL)



4 PRECISION

The precision of this assay was tested over 5 days in a morning and evening shift. On 3 of the 4 calibrated readers, 15 replicates of each of 3 levels were tested for a total of 45 replicates over the 3 readers. The data show the precision of the assay on the Theranos System

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

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

1.1.1 A total of 45 runs were performed over 5 days

1.1.2 The report will include scatter plots, precision summary tables and conclusions about the performance claim(s) regarding within-run (repeatability), between-day, between-lot and within laboratory precision.

1.1.3 Acceptance criteria: The %CV of replicates at each concentration of the samples should not be more than 20% (25% at LLOQ and ULOQ) for ELISA assays.

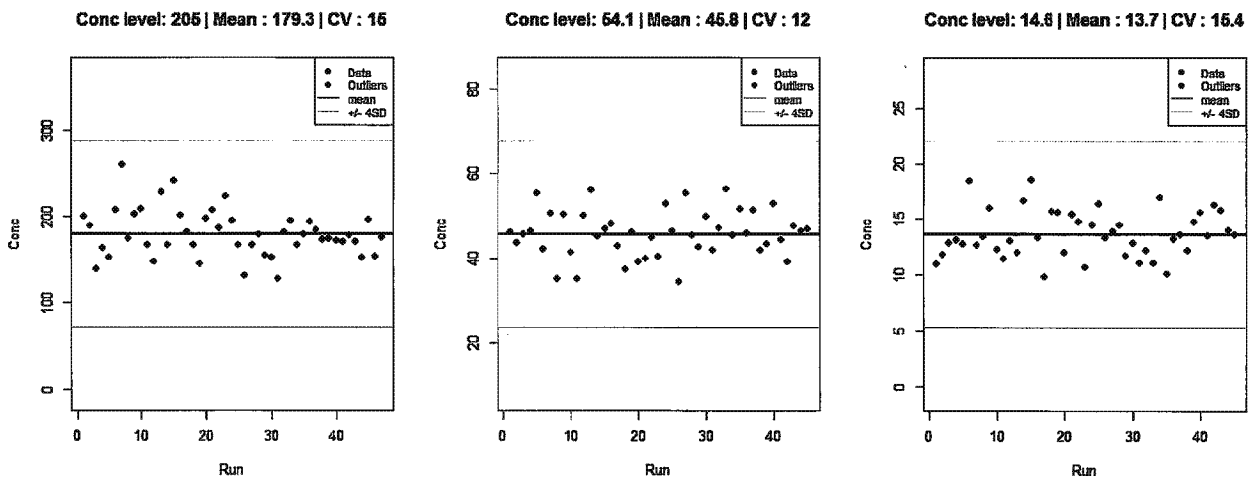
Table 4 : Acceptable Performance

Decision Level	TAE(%)
15 ng/mL	3.6 ng/mL or 30%
50 ng/mL	3.6 ng/mL or 30%
200 ng/mL	3.6 ng/mL or 30 %

Table 5 : Precision Summary

Medical Decision Levels	Prolactin [ng/mL]		Precision CV	Allowable Bias
	Nominal value	CLIA Concentration		
1	205	179	15%	15%
2	54.5	46	12%	18%
3	14.6	13.7	15%	15%

Figure 2: Precision Plot



5 ACCURACY/COMPARABILITY

5.1 To test the accuracy of the assay on the Theranos System, 100 unique patient samples were assayed using both the predicate method (Siemens Immulite) and the Theranos System. 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. (Results: Linear regression was used to fit Prolactin method comparison data. Correlation between Theranos results and Immulite results was determined by equation of the fit, y=1.0274x+0.5049..

Table 6: Clinical Correlation

Clia	Theranos	Clia	Theranos-Corr
197.7	207.1	197.7	201.1
47.4	45.3	47.4	43.6
60.5	58.2	60.5	56.2
223.2	243.7	223.2	236.7
40.4	36.1	40.4	34.6
50.7	50.0	50.7	48.2
43.5	47.5	43.5	45.7
30.6	33.3	30.6	32.0
15.6	17.2	15.6	16.2
2.9	3.5	2.9	2.9
5.8	6.6	5.8	5.9
2.3	2.7	2.3	2.2
3.1	3.9	3.1	3.3
9.3	8.3	9.3	7.6
13.2	12.9	13.2	12.1
16.1	11.5	16.1	10.8
8	10.8	8	10.0
13.6	12.3	13.6	11.5
9.8	12.3	9.8	11.5
9.4	10.8	9.4	10.0
6.6	9.1	6.6	8.3
54.6	47.1	54.6	45.3
7.3	7.5	7.3	6.8

21.6	23.2	21.6	22.1
9	8.8	9	8.0
13.6	13.3	13.6	12.5
11.8	13.3	11.8	12.4
50.7	36.8	50.7	35.3
46.6	50.3	46.6	48.5
36.6	35.9	36.6	34.5
146	132.7	146	128.7
61.2	49.5	61.2	47.7
85.6	95.2	85.6	92.2
62.2	71.6	62.2	69.2
149	137.7	149	133.5
82	91.2	82	88.3
65	65.7	65	63.4
58.5	116.7	58.5	113.1
35.9	49.3	35.9	47.5
11.05	9.0	11.05	8.3
6.76	6.1	6.76	5.5
16.15	16.4	16.15	15.5
3.33	3.6	3.33	3.0
4.36	5.0	4.36	4.3
12.2	11.9	12.2	11.1
3.66	3.7	3.66	3.1
6.81	9.0	6.81	8.3
8.24	11.0	8.24	10.2
6.73	6.2	6.73	5.5
12.9	15.1	12.9	14.2
3.81	4.7	3.81	4.1
4.29	3.5	4.29	2.9
55.8	46.0	55.8	44.3
6.42	5.4	6.42	4.7
17.25	17.6	17.25	16.6
8.35	8.0	8.35	7.3
6.79	14.7	6.79	13.9
4.9	6.1	4.9	5.4
9.16	8.8	9.16	8.1

Figure 3: Bias Correction plot

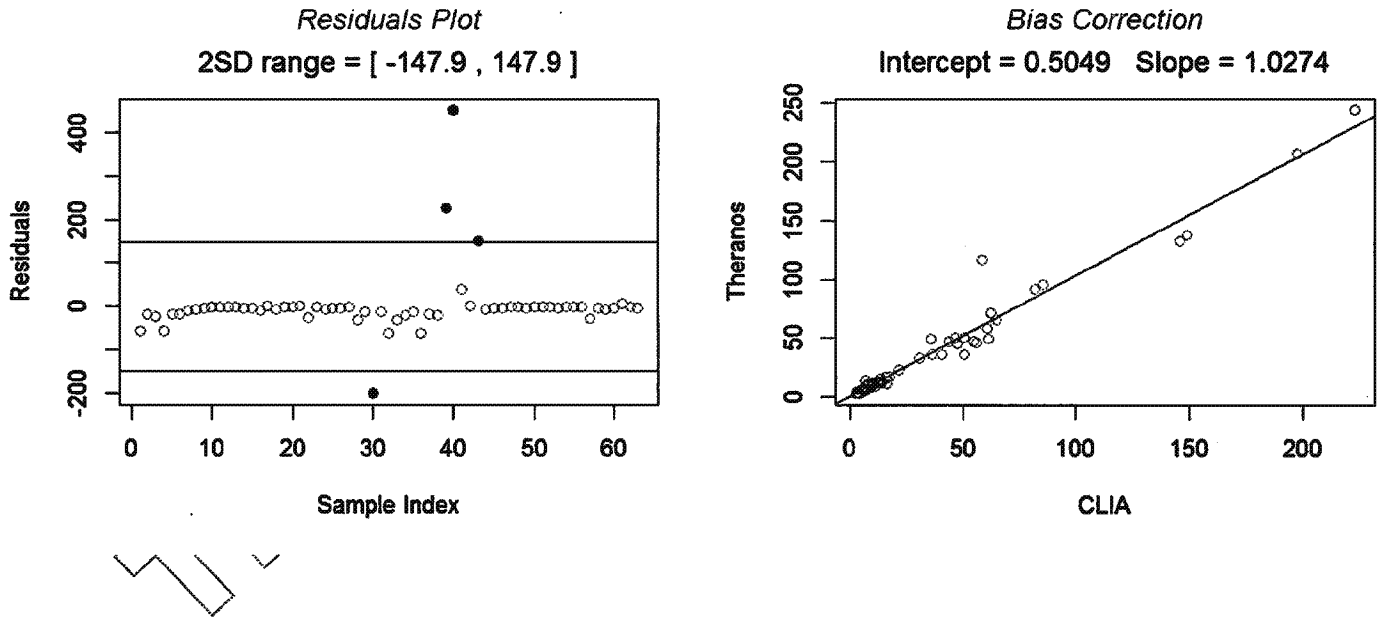


Figure 4: Scatter Plot CLSI guideline EP09-A2-IR section 4.2

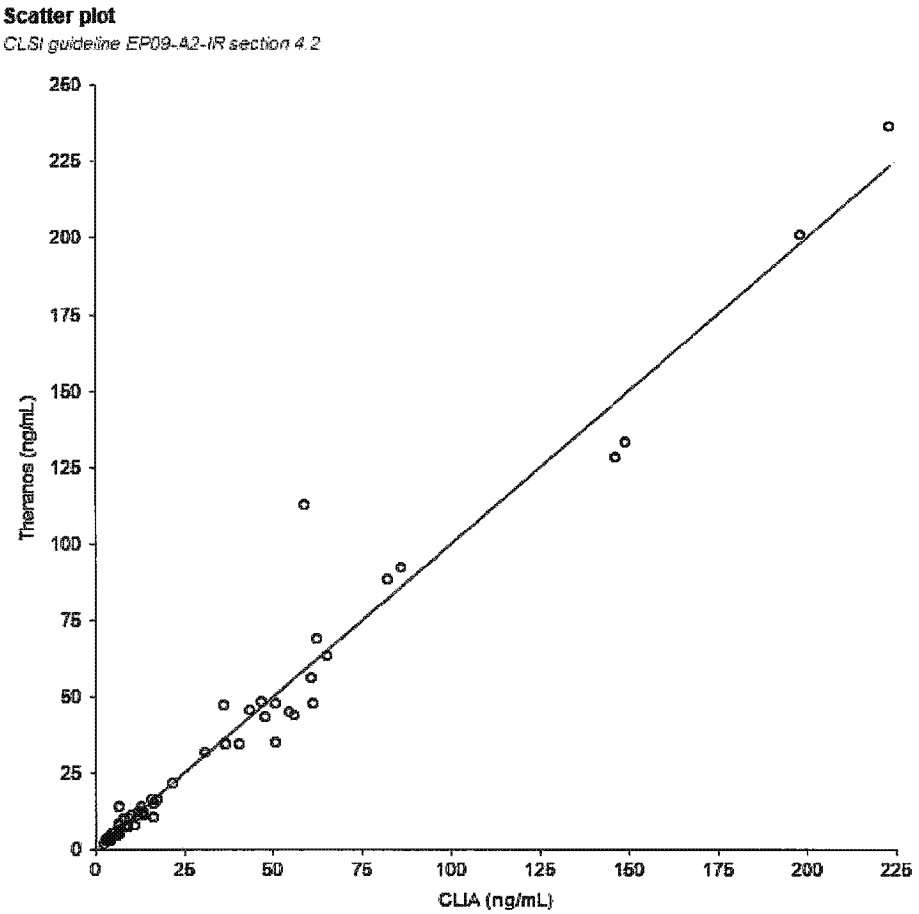
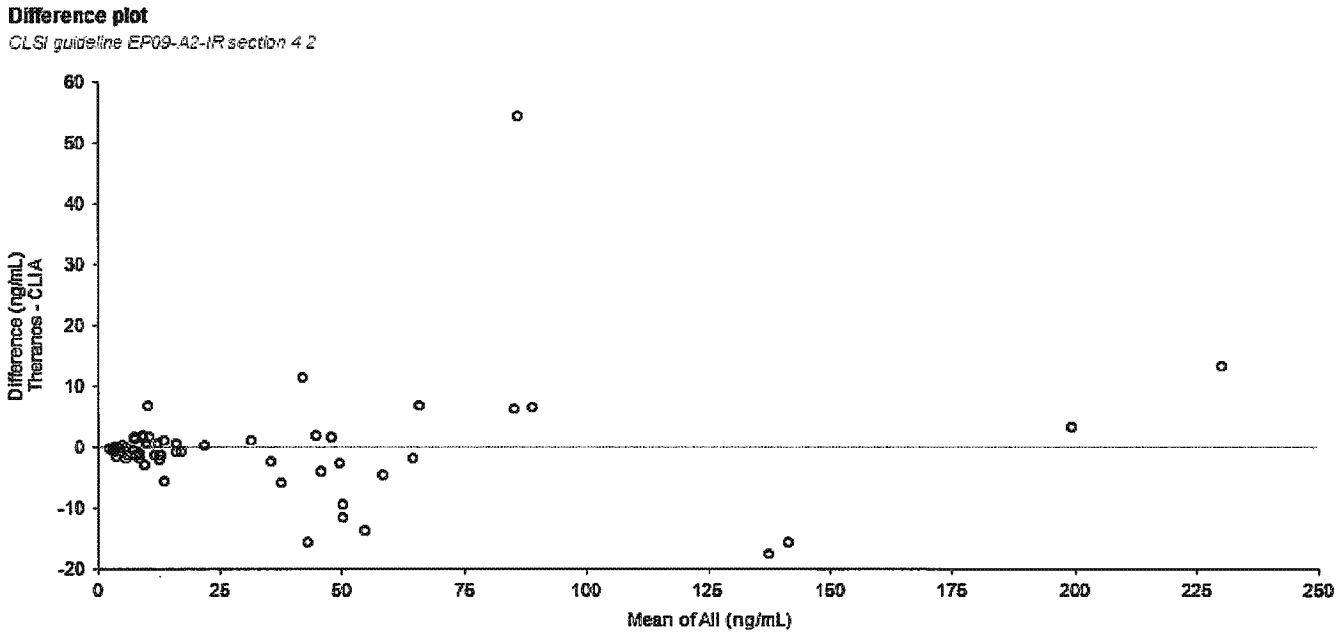


Figure 5: Difference Plot – CLSI Guideline EP09- A2-IR Section 4.2



Ordinary linear fit

CLSI guideline EP09-A2-IR section 5.1

n 59

Parameter	Estimate	SE	95% CI
Constant (Intercept)	-0.09947219	1.497241783	-3.097646996 to 2.89870
Proportional (slope)	1.003	0.0263	0.950 to 1.055

Sy.x 9.208228408

Comparability

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
1	15.00000000	-0.05646390	1.300133798	-2.359936933 to 2.54700	2.250000000
2	50.00000000	0.043888770	1.268825112	-2.496889890 to 2.58466	7.500000000
3	200.0000000	0.473971679	4.515645148	-8.508451741 to 9.51639	30.00000000

Difference is less than allowable bias: 15%.

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% - 14%
                     = 16%
```

As seen in figure , after correction, the mean bias is 3% , with a 95% confidence interval around it of [-5%, 11%]. This bias is within the total allowable bias, this validating the bias correction.

Figure 6: Verification of Bias Correction

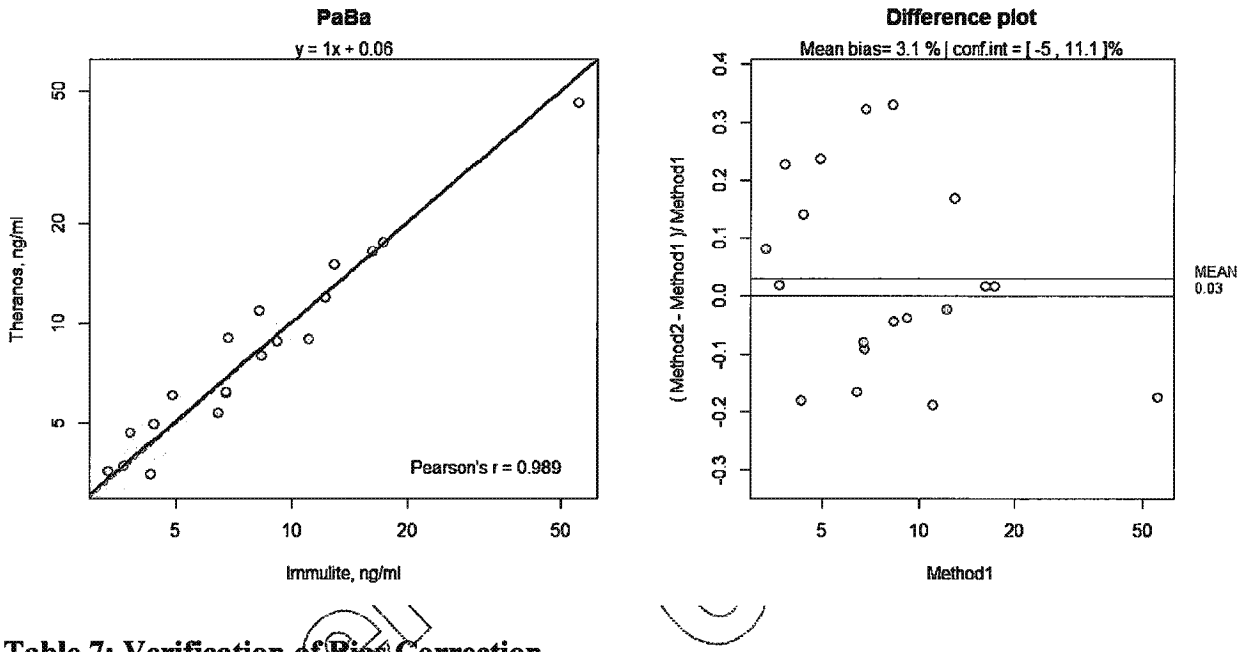


Table 7: Verification of Bias Correction

SampleID	Immulite	Theranos
Patient 1	11.1	9.0
Patient 2	6.8	6.1
Patient 3	16.2	16.4
Patient 4	3.3	3.6
Patient 5	4.4	5.0
Patient 6	12.2	11.9
Patient 7	3.7	3.7
Patient 8	6.8	9.0
Patient 9	8.2	11.0
Patient 10	6.7	6.2
Patient 11	12.9	15.1
Patient 12	3.8	4.7
Patient 13	4.3	3.5
Patient 14	55.8	46.0

Patient 15	6.4	5.4
Patient 16	17.3	17.6
Patient 17	8.4	8.0
Patient 18	6.8	14.7
Patient 19	4.9	6.1
Patient 20	9.2	8.8

6 DILUTION LINEARITY

A high analyte sample was selected and serially diluted using a pooled low sample to a total of 10 points to test the linearity. The reported values of each level were calculated based on the value of the high analyte sample as reported by the predicate method Siemens Immulite 2000. Apart from the top level, all the other levels had excellent recovery within 100 ± 20%.

6.1 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% (100 ± 25% at LLOQ and ULOQ standards) of their nominal value, and when plotted, the R^2 value should be equal or greater than 0.95.

6.2 CLSI guideline EP06-A will be followed to establish the measuring interval by performing a linearity study:

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

6.2.1.1 A scatter plot

6.2.1.2 Repeatability summary table showing the repeatability statistics for each concentration evaluated

6.2.1.3 Linearity summary table including the values obtained with a linear fit and the best-fitting nonlinear fit.

6.2.1.4 Conclusion regarding the measuring interval of the study

The response is linear over the measuring interval of the assay. Apart from the top level (High Patient sample-NEAT), all the other levels had excellent recovery within 100 ± 20%.

Table 8: Dilution Linearity

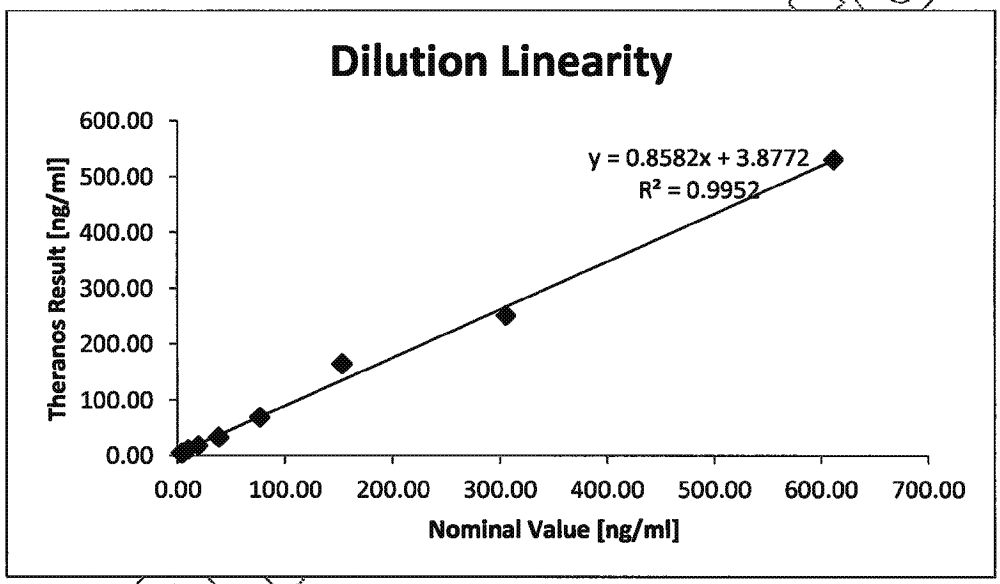
Reported Value [ng/mL]	All Tips		Dark Exclusion		Intra-Cart.		Inter-Cart.		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
1223	504236	617851	504236	617851	561044	14%	678025	22%	654.03	OORH	OORH	OORH	OORH
	910644	794780	910644	794780	852712	10%			OORH	OORH			
	611497	629140	611497	629140	620319	2%			OORH	OORH			
611.50	348184	380195	348184	380195	364190	6%	403082	10%	463.28	501.72	529.39	9%	87%
	441666	447970	441666	447970	444818	1%			576.45	584.19			
	384951	415524	384951	415524	400238	5%			507.46	544.51			
305.75	221380	192692	221380	192692	207036	10%	170152	20%	312.41	278.09	250.88	16%	82%
	143446	174085	143446	174085	158766	14%			218.18	255.65			
	128527	160784	128527	160784	144656	16%			199.61	239.47			
152.88	71861	88316	71861	88316	80089	15%	101313	20%	125.44	147.79	164.90	16%	108%
	109171	115477	109171	115477	112324	4%			175.05	183.12			
	94945	128105	94945	128105	111525	21%			156.57	199.08			
76.44	34427	35157	34427	35157	34792	1%	33913	20%	69.32	70.72	68.68	16%	90%
	39639	42044	39639	42044	40842	4%			77.96	81.75			
	26023	26188	26023	26188	26106	0%			55.18	55.48			
38.22	15436	14501	15436	14501	14969	4%	14133	12%	35.16	33.24	32.47	11%	85%
	15462	11070	15462	11070	13266	23%			35.21	25.87			
	13721	14607	13721	14607	14164	4%			31.61	33.46			
19.11	8887	8336	8887	8336	8612	5%	7426	19%	20.88	19.57	17.36	20%	91%
	6080	5371	6080	5371	5726	9%			13.98	12.14			
	8431	7451	8431	7451	7941	9%			19.80	17.42			
9.55	3879	5416	3879	5416	4648	23%	4740	18%	8.10	12.25	10.45	22%	109%
	3947	5945	3947	5945	4946	29%			8.28	13.63			
	4244	5011	4244	5011	4628	12%			9.10	11.18			
4.78	3246	3615	3246	3615	3431	8%	2792	20%	6.32	7.36	5.03	32%	105%
	2392	2959	2392	2959	2676	15%			3.88	5.50			
	2358	2184	2358	2184	2271	5%			3.79	3.29			
3.58	1730	1615					2516	10%	NA	NA	4.24	16%	118%
	2526	2858	2526	2858	2692	9%			4.27	5.22			
	2325	2356	2325	2356	2341	1%			3.69	3.78			

Table 9: Dilution Linearity Summary

Sample ID	Nominal Value [ng/ml]	Theranos Result [ng/ml]	% Recovery
High Patient Sample	1223	OORH	OORH
1/2	611.50	529.39	87%
1/4	305.75	250.88	82%
1/8	152.88	164.90	108%
1/16	76.44	68.68	90%

1/32	38.22	32.47	85%
1/64	19.11	17.36	91%
1/128	9.55	10.45	109%
1/256	4.78	5.03	105%
Low Patient Sample	3.58	4.24	118%

Figure 7: 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 adult females **1.9-25 nM** and adult males **2.5-17 nM**.
Samples were collected from 20 unique female donors and 9 male donors. These samples were confirmed via predicate method listed in section 1.2. Only 1 out of the 20 female subjects tested (sample # 16) had prolactin levels outside of the reference range (> 25 nM). Nine out of nine male subjects tested had prolactin levels within the reference range 2.5-17 nM.
Overall, 95 % of the patients tested fell within the reference range, and is acceptable for verification of the reference range.

Table 10: Reference Range verification

Sample ID	CLIA Reported	Theranos Corrected Venous EDTA
Patient 1	7.28	9.15
Patient 2	13.4	15.74
Patient 3	5.5	11.55
Patient 4	6.89	7.79
Patient 5	7.79	9.93
Patient 6	6.66	8.41
Patient 7	8.14	13.41
Patient 8	7.34	6.01
Patient 9	8.82	10.76
Patient 10	13	13.41
Patient 11	9.14	8.82
Patient 12	6.78	5.41
Patient 13	11	12.84
Patient 14	15.5	16.61
Patient 15	13.6	18.74

Patient 16	21	28.18
Patient 17	16.3	24.23
Patient 18	5.6	5.83
Patient 19	6.24	6
Patient 20	8.45	7

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 was performed using matched fingerstick blood and venous blood. The reference interval established for the predicate method is adult females **1.9-25 nM** and adult males 2.5-17 nM

8.1 Calculated concentrations are based on the mean RLU of 4 cartridges tested.

20 unique female donors donated 2 venous tubes of blood and 2 fingerstick samples.
19 unique male donors donated 2 venous tubes of blood and 2 fingerstick samples

Of 20 unique female samples tested 18 samples were within the reference range. 2 samples tested higher than 25nM (sample 11 and 13). Since the female subjects were not chosen based on a questionnaire and given that certain conditions like oral contraceptives can cause high levels of prolactin it is conceivable that samples could lie outside of the reference range.

All nineteen male subjects had prolactin levels well within the reference range of 2.5 -17 nM and passed the reference range verification test.

The response is linear over the measuring interval of the assay. There was excellent correlation between fingerstick and venous.

Table 11: Venous v. Fingerstick: 20 females

Sample Type	All Tips		Dark Exclu.		Intra-Cart.		Inter-Cart. (ng/ml)				Concentration (ng/ml)			Overal l
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	Mean
Venous Tube 1	9	3005	DARK	3005			3410	28%	3635	23%	NA	5.63	6.76	7.42
Venous Tube 2	2707	4519	2707	4519	3613	35%					4.78	9.86		
Fingerstick 1	3044	4762	3044	4762	3903	31%	3804	21%			5.75	10.5	7.86	

Fingerstick 2	4197	3212	4197	3212	3705	19%					8.97	6.22		
Venous Tube 1	11198	7873	11198	7873	9536	25%	8234	34%	8930	22%	26.2	18.5	19.14	20.98
Venous Tube 2	5632	86012	5632								12.8	NA		
Fingerstick 1	10152	9412	10152	9412	9782	5%	9626	5%			23.8	22.1	22.59	
Fingerstick 2	9313	628	9313								21.9	NA		
Venous Tube 1	6717	6372	6717	6372	6545	4%	6927	10%	6225	17%	15.6	14.7	16.11	14.35
Venous Tube 2	7693		7693	DARK							18	NA		
Fingerstick 1	4920	4670	4920	4670	4795	4%	5698	19%			10.9	10.3	12.95	
Fingerstick 2	6793	6408	6793	6408	6601	4%					15.8	14.8		
Venous Tube 1	22	6968	DARK				4158	28%	4020	22%	NA	NA	8.84	8.49
Venous Tube 2	3340	4976	3340	4976	4158	28%					6.58	11.1		
Fingerstick 1	8978	8768					3881	24%			NA	NA	8.08	
Fingerstick 2	4540	3222	4540	3222	3881	24%					9.91	6.25		
Venous Tube 1	4424	2220		2220			2518	12%	2283	13%	NA	3.39	4.25	3.57
Venous Tube 2	2824	2511	2824	2511	2668	8%					5.12	4.22		
Fingerstick 1	2135	2022	2135	2022	2079	4%	2106	4%			3.15	2.84	3.07	
Fingerstick 2	2194	2072	2194	2072	2133	4%					3.32	2.98		
Venous Tube 1	10770	5537		5537			4569	20%	4522	23%	NA	12.6	9.96	9.86
Venous Tube 2	3758	4411	3758	4411	4085	11%					7.76	9.56		
Fingerstick 1	3051	3881	3051	3881	3466	17%	4487	28%			5.77	8.1	9.71	
Fingerstick 2	5188	5829	5188	5829	5509	8%					11.7	13.3		
Venous Tube 1	5978	4997	5978	4997	5488	13%	5097	16%	4896	24%	13.7	11.1	11.39	10.87
Venous Tube 2	2556	4317		4317							NA	9.3		
Fingerstick 1	3360	3824	3360	3824	3592	9%	4744	32%			6.64	7.94	10.38	
Fingerstick 2	5022	6771	5022	6771	5897	21%					11.2	15.7		
Venous Tube 1	8152	9307					4644	14%	4734	8%	NA	NA	10.18	10.44
Venous Tube 2	4176	5111	4176	5111	4644	14%					8.92	11.5		
Fingerstick 1	4730	4998	4730	4998	4864	4%	4780	5%			10.4	11.2	10.56	

Fingerstick 2	4940	4450	4940	4450	4695	7%					11	9.67		
Venous Tube 1	1409	2151		2151			2205	3%	2592	19%	NA	3.2	3.35	4.45
Venous Tube 2	2266	2199	2266	2199	2233	2%					3.53	3.34		
Fingerstick 1	787	2689		2689			2978	12%			NA	4.73	5.56	
Fingerstick 2	2875	3371	2875	3371	3123	11%					5.26	6.67		
Venous Tube 1	7232	7335	7232	7335	7284	1%	8462	19%	9014	34%	16.9	17.1	19.8	21.18
Venous Tube 2	8543	10736	8543	10736	9640	16%					20.1	25.1		
Fingerstick 1	15291	2	15291	DARK			9751	49%			34.9	NA	22.46	
Fingerstick 2	7026	6937	7026	6937	6982	1%					16.4	16.2		
Venous Tube 1	11669	8	11669	DARK			11275	5%	11375	6%	27.2	NA	26.32	26.55
Venous Tube 2	3092	10881		10881							NA	25.5		
Fingerstick 1	10735	12214	10735	12214	11475	9%	11475	9%			25.1	28.4	26.75	
Fingerstick 2	5881	6447									NA	NA		
Venous Tube 1	6846	8288	6846	8288	7567	13%	8374	21%	8664	17%	15.9	19.5	19.58	20.35
Venous Tube 2	10817	7543	10817	7543	9180	25%					25.3	17.7		
Fingerstick 1	8769	10864	8769	10864	9817	15%	8954	15%			20.6	25.4	20.99	
Fingerstick 2	8033	8148	8033	8148	8091	1%					18.8	19.1		
Venous Tube 1	50130	33664	50130	33664	41897	28%	43943	17%	40177	21%	94.2	68.3	84.52	78.81
Venous Tube 2	48717	43260	48717	43260	45989	8%					92	83.7		
Fingerstick 1	45996	40711	45996	40711	43354	9%	36411	23%			87.9	79.7	72.48	
Fingerstick 2	29125	29812	29125	29812	29469	2%					60.6	61.8		
Venous Tube 1			DARK	DARK			3394	37%	3724	21%	NA	NA	6.71	7.66
Venous Tube 2	4292	2495	4292	2495	3394	37%					9.24	4.18		
Fingerstick 1	4538	4112	4538	4112	4325	7%	3889	15%			9.91	8.74	8.11	
Fingerstick 2	3702	3204	3702	3204	3453	10%					7.6	6.2		
Venous Tube 1	5349	4698	5349	4698	5024	9%	4630	23%	4466	20%	12.1	10.3	10.11	9.71
Venous Tube 2	5390	3083	5390	3083	4237	39%					12.2	5.86		
Fingerstick 1	9	12	DARK	DARK			4137	12%			NA	NA	8.8	

Fingerstick 2	3778	4496	3778	4496	4137	12%					7.81	9.79		
Venous Tube 1	3754	6868	3754	6868	5311	41%	6038	17%			6	9.6	8.3	8.7
Venous Tube 2	5395	8134	5395	8134	6765	29%					8.4	9		
Fingerstick 1	3885	6763	3885	6763	5324	38%	6892	32%			8.4	7.2	9.2	
Fingerstick 2	7613	9307	7613	9307	8460	14%					11.2	10.1		
Venous Tube 1	6189	3888	6189	3888	5039	32%	4785	25%	4678	28%	14.3	8.12	10.52	10.29
Venous Tube 2	3750	5313	3750	5313	4532	24%					7.74	12		
Fingerstick 1	6271	4630	6271	4630	5451	21%	4572	35%			14.5	10.2	9.91	
Fingerstick 2	2428	4958	2428	4958	3693	48%					3.99	11		
Venous Tube 1	2181	2660	2181	2660	2421	14%	2463	9%	2662	15%	3.28	4.65	4.09	4.66
Venous Tube 2	2646	2364	2646	2364	2505	8%					4.61	3.8		
Fingerstick 1	2783	3340	2783	3340	3062	13%	3062	13%			5	6.58	5.79	
Fingerstick 2	3978			DARK							NA	NA		
Venous Tube 1	5723	7405	5723	7405	6564	18%	5873	25%	6024	20%	13.1	17.3	13.38	13.84
Venous Tube 2	3624	4490		4490							NA	9.78		
Fingerstick 1	7512	3199	7512				6175	20%			17.6	NA	14.18	
Fingerstick 2	5868	5146	5868	5146	5507	9%					13.4	11.5		
Venous Tube 1	6953	4477	6953	4477	5715	31%	5811	18%	5743	14%	16.2	9.74	13.25	13.11
Venous Tube 2	5884	5928	5884	5928	5906	1%					13.5	13.6		
Fingerstick 1	5979	5237	5979	5237	5608	9%	5608	9%			13.7	11.8	12.75	
Fingerstick 2	2083	2378									NA	NA		

Table 12: Venous v. Fingerstick Comparison Summary: 20 Females

Sample ID	Corrected	
	Venous Tube	Fingerstick
Patient 1	7.45	8.66
Patient 2	21.06	24.85
Patient 3	17.73	14.26
Patient 4	9.73	8.9

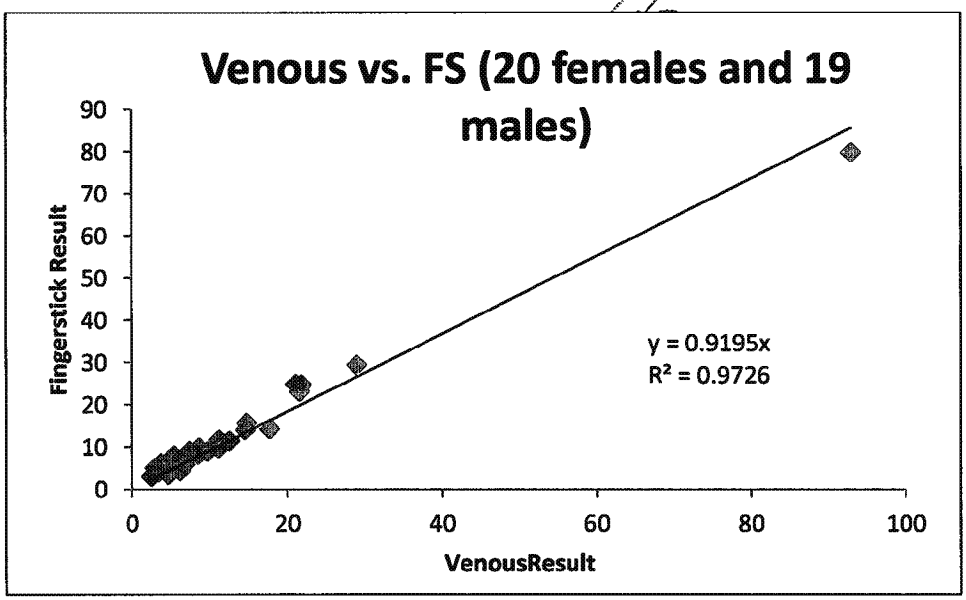
Patient 5	4.69	3.4
Patient 6	10.97	10.69
Patient 7	12.54	11.43
Patient 8	11.21	11.62
Patient 9	3.71	6.13
Patient 10	21.78	24.71
Patient 11	28.95	29.42
Patient 12	21.54	23.09
Patient 13	92.91	79.67
Patient 14	7.39	8.94
Patient 15	11.14	9.7
Patient 16	6.8	5.9
Patient 17	11.59	10.92
Patient 18	4.51	6.39
Patient 19	14.73	15.61
Patient 20	14.58	14.03

Table 13: Venous v. Fingerstick Comparison Summary: 19 Male Subjects

Sample ID	Corrected	
	Venous Tube	Fingerstick
Patient 1	8.5	9.7
Patient 2	3.4	4.1
Patient 3	2.5	3
Patient 4	8.5	8.2
Patient 5	8.6	9.8
Patient 6	5.5	5
Patient 7	7.1	7.6
Patient 8	4.4	4.2
Patient 9	4.3	4.2
Patient 10	5.4	7.9
Patient 11	6.2	4.3
Patient 12	4.8	5.3
Patient 13	2.9	5.0

Patient 14	3.3	4.8
Patient 15	7.2	8.0
Patient 16	6.7	7.6
Patient 17	5.4	4.9
Patient 18	6.9	6.9
Patient 19	5.4	6.1

Figure 8: Venous (Corrected) v. Fingerstick (Corrected) Comparison Graph



9 ANALYTICAL SENSITIVITY

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

9.1 Verification of Limit of Blank

9.1.1 Acceptance Criteria: the percentage of 60 blank replicates that return OORL results should be greater than 90%, or the percentage of 20 blank replicates should be greater than 85%.

9.1.2 When the results were grouped into 20 replicates of 3 cartridges, 90% returned OORL results.

9.2 Verification of Limit of Detection

9.2.1 Acceptance Criteria: the percentage of 60 LLOQ replicates that return quantifiable results should be greater than 90%, or the percentage of 20 LLOQ replicates should be greater than 85%.

9.2.2 By grouping the results into 20 replicates of 3 cartridges, 95% returned quantifiable results

9.3 20 replicates of LLOQ were tested. If 80% of replicates at this level were reportable, the sample was diluted 1:1 and 20 replicates of the 1/2xLLOQ sample were tested. If 80% of these replicates were reportable, it was again diluted 1:1 and tested. This was repeated until less than 80% of the replicates were not reportable (OORL).

9.3.1 Of 20 replicates (calculated by cartridge) at 1/2xLLOQ, 70% returned quantifiable results.

9.3.2 Of 20 replicates (calculated by cartridge) at 1/4LLOQ, 100% returned non-quantifiable results.

Table 14: Blank Replicates

All Tips		Dark Exclusion		Intra-Cartridge			Inter-Cartridge		
Tip1	Tip2	Tip1	Tip2	Mean	%CV	Result	Mean	%CV	Result
846	795	846	795	821	4%	OORL	1056	28%	OORL
1683	883		883	883		OORL			
1400	1357	1400	1357	1379	2%	1.11			

1322	1196	1322	1196	1259	7%	OORL			
1623	1212	1623	1212	1418	21%	1.21	1276	15%	
1064	1239	1064	1239	1152	11%	OORL			OORL
1407	2013	1407				NA			
1056	12	1056	DARK	1056		OORL	1225	14%	
638	1213		1213	1213		OORL			OORL
775	1	775	DARK	775		OORL			
925	1602	925	1602	1264	38%	OORL	1190	34%	
2090	1456		1456	1456		1.31			OORL
948	837	948	837	893	9%	OORL			
1013	1000	1013	1000	1007	1%	OORL	1049	16%	
1194	1302	1194	1302	1248	6%	OORL			OORL
1184	1452	1184	1452	1318	14%	OORL			
909	1118	909	1118	1014	15%	OORL	1199	15%	
1218	1313	1218	1313	1266	5%	OORL			OORL
1042	584	1042		1042		OORL			
1431	1707	1431		1431		1.24	1246	13%	
1259	1250	1259	1250	1255	1%	OORL			OORL
1008	973	1008	973	991	2%	OORL			
1432	1403	1432	1403	1418	1%	1.21	1080	25%	
879	786	879	786	833	8%	OORL			OORL
1	670	DARK	670						
1904	1001		1001	1001		OORL	1085	29%	
1325	1345	1325	1345	1335	1%	OORL			OORL
1400	2336	1400		1400		1.17			
1505	1308	1505	1308	1407	10%	1.18	1408	6%	
1839	1420		1420	1420		1.22			1.19
1355	1614	1355		1355		1.06			
1329	1292	1329	1292	1311	2%	OORL	1312	16%	
992	1594	992	1594	1293	33%	OORL			OORL
1300	1214	1300	1214	1257	5%	OORL			
1527	1527	1527		1527		1.49	1328	12%	
1154	1444	1154	1444	1299	16%	OORL			OORL
1336	1292	1336	1292	1314	2%	OORL			
1322	2153	1322		1322		OORL	1378	6%	
1486	1454	1486	1454	1470	2%	1.34			1.11
4851	929		929	929		OORL			
1164	1397	1164	1397	1281	13%	OORL	1291	20%	
1591	1374	1591	1374	1483	10%	1.37			OORL
1467	1371	1467	1371	1419	5%	1.21			
1559	1714	1559	1714	1637	7%	1.78	1322	26%	
814	1005	814	1005	910	15%	OORL			OORL
1654	1359	1654	1359	1507	14%	1.44			
1141	986	1141	986	1064	10%	OORL	1245	19%	
1209	1118	1209	1118	1164	6%	OORL			OORL
1084	1001	1084	1001	1043	6%	OORL	1296	24%	OORL

1103	1229	1103	1229	1166	8%	OORL			
1579	1780	1579	1780	1680	8%	1.89			
1027	1282	1027	1282	1155	16%	OORL			
674	740	674	740	707	7%	OORL	952	26%	
487	1038		1038	1038		OORL			OORL
874	981	874	981	928	8%	OORL			
1372	1313	1372	1313	1343	3%	1.03	1214	19%	
1364	1377	1364	1377	1371	1%	1.09			OORL
863	1059	863	1059	961	14%	OORL			
1572	1153	1572	1153	1363	22%	1.07	1163	20%	
1245	1088	1245	1088	1167	10%	OORL			OORL

Table 15: LLOQ Replicates

All Tips		Dark Exclusion		Intra-Cartridge			Inter-Cartridge		
Tip1	Tip2	Tip1	Tip2	Mean	%CV	Result	Mean	%CV	Result
801	1477	801	1477	1139	42%	OORL			
38	325105	DARK				NA	1654	33%	
747	1189	747	1189	968	32%	OORL			OORL
4207	4987					NA			
1753	2371	1753	2371	2062	21%	2.95	1654	32%	
1306	1186	1306	1186	1246	7%	OORL			1.82
2410	2088	2410	2088	2249	10%	3.48			
1624	2064	1624	2064	1844	17%	2.34	1880	22%	
1848	1248	1848	1248	1548	27%	1.54			2.44
1177	1344	1177	1344	1261	9%	OORL			
1458	1521	1458	1521	1490	3%	1.39	1504	21%	
2019	893	2019		2019		2.83			1.43
1569	1721	1569	1721	1645	7%	1.80			
1817	1571	1817	1571	1694	10%	1.93	1620	9%	
3344	1421		1421	1421		1.22			1.73
1485	3369	1485		1485		1.38			
1750	1644	1750	1644	1697	4%	1.94	1567	11%	
1642	1312	1642	1312	1477	16%	1.36			1.59
2391	2353	2391	2353	2372	1%	3.83			
642	557					NA	2142	16%	
1641	2184	1641	2184	1913	20%	2.53			3.17
1483	2047	1483	2047	1765	23%	2.12			
1451	4	1451	DARK	1451		1.29	1621	18%	
2716	1503		1503	1503		1.43			1.74
1501	1479	1501	1479	1490	1%	1.39			
1201	11	1201	DARK	1201		OORL	1611	28%	
2322	2262		2262	2262		3.51			1.71
2591	1743		1743	1743		2.06			
2287	2448	2287		2287		3.59	1862	17%	
1856	1563	1856	1563	1710	12%	1.97			2.39

5	13	DARK	DARK			NA			
1978	2257	1978	2257	2118	9%	3.10	2118	9%	
543	515					NA			3.10
1790	2298	1790	2298	2044	18%	2.90			
1323	3493	1323		1323		OORL	1773	23%	
1681	9	1681	DARK	1681		1.90			2.15
1749	6	1749	DARK	1749		2.08			
1504	27523	1504		1504		1.43	1506	12%	
1427	1343	1427	1343	1385	4%	1.13			1.43
1118	1593	1118	1593	1356	25%	1.06			
595	1392		1392	1392		1.15	1459	31%	
1029	2165	1029	2165	1597	50%	1.67			1.32
1524	1276	1524	1276	1400	13%	1.17			
1424	1468	1424	1468	1446	2%	1.28	1430	6%	
1413	1472	1413	1472	1443	3%	1.27			1.24
1608	835	1608		1608		1.70			
2438	1724		1724	1724		2.01	1551	15%	
1655	1215	1655	1215	1435	22%	1.25			1.55
14	18	DARK	DARK			NA			
1677	1737	1677	1737	1707	2%	1.97	1497	17%	
1344	1228	1344	1228	1286	6%	OORL			1.41
1329	3723	1329		1329		OORL			
1618	1962	1618	1962	1790	14%	2.19	1523	18%	
1415	1289	1415	1289	1352	7%	1.05			1.48
1593	1467	1593	1467	1530	6%	1.50			
1947	1647	1947	1647	1797	12%	2.21	1530	18%	
1378	1149	1378	1149	1264	13%	OORL			1.50
1649	1477	1649	1477	1563	8%	1.58			
2151	2272	2151	2272	2212	4%	3.37	1784	19%	
1477	1677	1477	1677	1577	9%	1.62			2.17

Table 16: ½ LLOQ Replicates

All Tips		Dark Exclusion		Intra-Cartridge		
Tip1	Tip2	Tip1	Tip2	Mean	%CV	Result
1036	1075	1036	1075	1056	3%	OORL
1641	1241	1641	1241	1441	20%	1.27
1069	1247	1069	1247	1158	11%	OORL
1497	1974	1497	1974	1736	19%	2.04
1879	1262	1879	1262	1571	28%	1.60
1310	1339	1310	1339	1325	2%	OORL
1763	1293	1763	1293	1528	22%	1.49
1246	1455	1246	1455	1351	11%	1.04
1595	1816	1595	1816	1706	9%	1.96

1280	1145	1280	1145	1213	8%	OORL
1511	1504	1511	1504	1508	0%	1.44
1494	1716	1494	1716	1605	10%	1.69
1695	1434	1695	1434	1565	12%	1.59
1596	1549	1596	1549	1573	2%	1.61
1820	1647	1820	1647	1734	7%	2.04
1179	1026	1179	1026	1103	10%	OORL
1573	3892	1573		1573		1.61
1691	1522	1691	1522	1607	7%	1.70
1513	1468	1513	1468	1491	2%	1.39
1046	916	1046	916	981	9%	OORL

Table 17: 1/4 LLOQ Replicates

All Tips		Dark Exclusion		Intra-Cartridge		
Tip1	Tip2	Tip1	Tip2	Mean	%CV	Result
1097	1047	1097	1047	1072	3%	OORL
821	1162	821	1162	992	24%	OORL
988	858	988	858	923	10%	OORL
881	695	881	695	788	17%	OORL
1239	1292	1239	1292	1266	3%	OORL
954	967	954	967	961	1%	OORL
1377	1247	1377	1247	1312	7%	OORL
772	740	772	740	756	3%	OORL
1145	1022	1145	1022	1084	8%	OORL
559	690	559	690	625	15%	OORL
1115	914	1115	914	1015	14%	OORL
990	1024	990	1024	1007	2%	OORL
1081	1293		1293	1293		OORL
1686	1240		1240	1240		OORL
808	20	808	DARK	808		OORL
1103	1137	1103	1137	1120	2%	OORL
1527	1050	1527	1050	1289	26%	OORL
999	865	999	865	932	10%	OORL
852	807	852	807	830	4%	OORL
1157	923	1157	923	1040	16%	OORL

Table 18: Analytical Sensitivity Summary

Blank Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% OORL	Mean	%CV
Intra-Cartridge	58	**1214**	**18%**	**OORL**	**67%**	*1.29*	*18%*
Inter-Cartridge	20	**1216**	**10%**	**OORL**	**90%**	*1.15*	*5%*

LLOQ Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% Quantifiable	Mean	%CV
Intra-Cartridge	54	**1621**	**20%**	**1.73637025**	**83%**	*1.97*	*39%*
Inter-Cartridge	20	**1634**	**15%**	**1.77058363**	**95%**	*1.86*	*31%*

1/2 LLOQ Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% OORL	Mean	%CV
Intra-Cartridge	20	**1441**	**16%**	**1.268624602**	**70%**	*1.61*	*18%*

1/4 LLOQ Summary	N	RLUs		Calculated From Mean	Result		
		Mean	%CV		% OORL	Mean	%CV
Intra-Cartridge	20	**1017**	**19%**	**OORL**	**100%**		

10 ANTICOAGULANT COMPARISON

To test the effect of anticoagulants, 20 unique patients donated a total of 3 tubes, one EDTA, one Li Heparin, and one Serum.

Table 19: EDTA Plasma

Reported Value [ng/mL]	All Tips		Dark Exclu.		Intra-Cart.		Inter-Cart.		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
7.28	6910	4261		4261			3954	15%	NA	9.15	8.30	19%	114%
	3214	3479	3214	3479	3347	6%			6.23	6.98			
	4576	4238	4576	4238	4407	5%			10.01	9.09			
13.4	7436	7084	7436	7084	7260	3%	6207	16%	17.39	16.52	14.30	18%	107%
	1615	5934		5934					NA	13.60			
	5191	5388	5191	5388	5290	3%			11.66	12.18			
5.5	4466	3232	4466	3232	3849	23%	4754	23%	9.71	6.28	10.49	28%	191%
	4870	4992	4870	4992	4931	2%			10.80	11.13			
	6212	16	6212	DARK					14.32	NA			
6.89	3587	3985	3587	3985	3786	7%	3511	21%	7.28	8.39	7.07	29%	103%
	4367	3797	4367	3797	4082	10%			9.44	7.87			
	2939	2389	2939	2389	2664	15%			5.45	3.88			
7.79	1044	2920		2920			4211	20%	NA	5.39	9.01	26%	116%
	3823	4520	3823	4520	4172	12%			7.94	9.86			

	4835	4955	4835	4955	4895	2%			10.71	11.03			
6.66	3574	3698	3574	3698	3636	2%	3713	11%	7.24	7.59	7.63	15%	115%
	3156	3821	3156	3821	3489	13%			6.06	7.93			
	4314	7	4314	DARK					9.30	NA			
8.14	3813	6203	3813	6203	5008	34%	5390	22%	7.91	14.30	12.18	25%	150%
			DARK	DARK					NA	NA			
	5217	6326	5217	6326	5772	14%			11.73	14.61			
7.34	3649	3231	3649	3231	3440	9%	2941	23%	7.45	6.28	5.45	35%	74%
	6767	5581							NA	NA			
	2819	2063	2819	2063	2441	22%			5.10	2.95			
8.82	2395	4456		4456			4490	21%	NA	9.68	9.78	26%	111%
	3895	3785	3895	3785	3840	2%			8.14	7.83			
	5823	6880	5823						13.32	NA			
13	0	5512	DARK	5512			5391	2%	NA	12.51	12.19	2%	94%
			DARK	DARK					NA	NA			
	5335	5325	5335	5325	5330	0%			12.04	12.01			
9.14	4283	10	4283	DARK			3847	20%	9.21	NA	8.01	27%	88%
	4523	4146	4523	4146	4335	6%			9.87	8.83			
	3674	2609	3674	2609	3142	24%			7.52	4.50			
6.78	3827	2421	3827	2421	3124	32%	2748	24%	7.95	3.97	4.90	39%	72%
	2926	2453	2926	2453	2690	12%			5.41	4.06			
	1722	2112		2112					NA	3.09			
11	5804	4519	5804	4519	5162	18%	5193	20%	13.27	9.86	11.66	24%	106%
	3943	5065	3943	5065	4504	18%			8.27	11.32			
	6634	8	6634	DARK					15.39	NA			
15.5	6163	5739	6163	5739	5951	5%	6517	15%	14.19	13.10	15.09	16%	97%
	6781	8100	6781	8100	7441	13%			15.76	19.00			
	6931	5388	6931	5388	6160	18%			16.14	12.18			
13.6	6233	3751	6233				7294	13%	14.37	NA	17.04	14%	125%
	10604	9121							NA	NA			
	7507	8142	7507	8142	7825	6%			17.56	19.10			
21	8553	8443	8553	8443	8498	1%	10959	26%	20.09	19.82	25.63	24%	122%
	9825	7	9825	DARK					23.06	NA			
	13946	14030	13946	14030	13988	0%			32.08	32.26			
16.3	643	853					9382	11%	NA	NA	22.03	11%	135%
	8732	10380	8732	10380	9556	12%			20.51	24.32			
	10167	8248	10167	8248	9208	15%			23.84	19.36			
5.6	3185	3285	3185	3285	3235	2%	2881	20%	6.15	6.43	5.28	32%	94%
	2255	2233	2255	2233	2244	1%			3.49	3.43			
	3447	4	3447	DARK					6.89	NA			
6.24	2379	3300	2379	3300	2840	23%	2938	14%	3.85	6.47	5.44	21%	87%
	3380	3107	3380	3107	3244	6%			6.70	5.92			
	2860	2599	2860	2599	2730	7%			5.22	4.48			
8.45	3913	3487	3913	3487	3700	8%	3256	22%	8.19	7.00	6.35	32%	75%
	1164	4698							NA	NA			

	3379	2246	3379	2246	2813	28%			6.69	3.47			

Table 20: Li Heparin Plasma

Reported Value [ng/mL]	All Tips		Dark Exclu.		Intra-Cart.		Inter-Cart.		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
7.28	3475	3401	3475	3401	3438	2%	3244	23%	6.97	6.76	6.31	33%	87%
	4301	3316	4301	3316	3809	18%			9.26	6.52			
	2047	2924	2047	2924	2486	25%			2.91	5.40			
13.4	4731	6361	4731	6361	5546	21%	6736	21%	10.43	14.70	15.65	23%	117%
	6454	6365	6454	6365	6410	1%			14.93	14.71			
	9117	7388	9117	7388	8253	15%			21.42	17.27			
5.5	3567	2621	3567	2621	3094	22%	4016	28%	7.22	4.54	8.47	37%	154%
	6062	4970		4970					NA	11.07			
	6651	4905		4905					NA	10.90			
6.89	3100	2053	3100	2053	2577	29%	2546	16%	5.90	2.92	4.32	27%	63%
	2619	2081	2619	2081	2350	16%			4.53	3.00			
	2567	2858	2567	2858	2713	8%			4.38	5.22			
7.79	2643	3	2643	DARK			3759	21%	4.60	NA	7.76	28%	100%
	4575	3938	4575	3938	4257	11%			10.01	8.26			
	3315	4323	3315	4323	3819	19%			6.51	9.32			
6.66	2777	3503	2777	3503	3140	16%	3838	25%	4.98	7.04	7.98	33%	120%
	3243	1	3243	DARK					6.31	NA			
	4932	4733	4932	4733	4833	3%			10.97	10.43			
8.14	2041	3074					6916	6%	NA	NA	16.10	6%	198%
	6873	7188	6873	7188	7031	3%			15.99	16.77			
	7259	6345	7259	6345	6802	10%			16.95	14.66			
7.34	3570	3438	3570	3438	3504	3%	3478	25%	7.23	6.86	6.97	34%	95%
	2878	2647	2878	2647	2763	6%			5.27	4.61			
	4857	1591	4857						10.77	NA			
8.82	4034	5055	4034	5055	4545	16%	4862	15%	8.53	11.30	10.78	18%	122%
	4216	6101	4216	6101	5159	26%			9.03	14.03			
	4933	4832	4933	4832	4883	1%			10.97	10.70			
13	7825	5733	7825	5733	6779	22%	5836	24%	18.34	13.08	13.35	27%	103%
	4693	5091	4693	5091	4892	6%			10.33	11.39			
	3538	2726							NA	NA			
9.14	2336	2261	2336	2261	2299	2%	3080	26%	3.72	3.51	5.85	38%	64%
	3217	8251	3217						6.24	NA			
	4136	3449	4136	3449	3793	13%			8.81	6.89			
6.78	3208	2499	3208	2499	2854	18%	3312	17%	6.21	4.19	6.50	25%	96%
	3857	6419	3857						8.03	NA			
	3860	3135	3860	3135	3498	15%			8.04	6.00			
11	3176	3615	3176	3615	3396	9%	4278	20%	6.12	7.36	9.20	26%	84%
	5537	4654	5537	4654	5096	12%			12.57	10.22			
	3870	4817	3870	4817	4344	15%			8.07	10.66			

15.5	9159	7349	9159	7349	8254	16%	6765	21%	21.51	17.17	15.72	23%	101%
	5529	5164	5529	5164	5347	5%			12.55	11.59			
	6631	6757	6631	6757	6694	1%			15.38	15.70			
13.6	7836	7147	7836	7147	7492	7%	7120	18%	18.36	16.67	16.61	19%	122%
	8080	3873	8080						18.95	NA			
	4933	7604	4933	7604	6269	30%			10.97	17.80			
21	8840	13730	8840				7730	18%	20.77	NA	18.11	19%	86%
	6446	6011	6446	6011	6229	5%			14.91	13.80			
	8718	8634	8718	8634	8676	1%			20.48	20.28			
16.3	7051	9026	7051	9026	8039	17%	7896	15%	16.43	21.20	18.51	16%	114%
	6172	7397	6172	7397	6785	13%			14.22	17.29			
	8858	8873	8858	8873	8866	0%			20.81	20.84			
5.6			DARK	DARK			2323	16%	NA	NA	3.69	28%	66%
	2093	2134	2093	2134	2114	1%			3.04	3.15			
	3812	2743		2743					NA	4.89			
6.24	2694	4344	2694	4344	3519	33%	3533	23%	4.75	9.38	7.13	32%	114%
	4391	2657	4391	2657	3524	38%			9.51	4.64			
	3132	3980	3132	3980	3556	17%			6.00	8.38			
8.45	2743	2604	2743	2604	2674	4%	2889	12%	4.89	4.49	5.30	19%	63%
	2698	5308	2698						4.76	NA			
	2894	3504	2894	3504	3199	13%			5.32	7.05			

Table 21: Serum

Reported Value [ng/mL]	All Tips		Dark Exclu.		Intra-Cart.		Inter-Cart.		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
7.28	3164	3222	3164	3222	3193	1%	3624	15%	6.09	6.25	7.38	20%	101%
	4130	4287	4130	4287	4209	3%			8.79	9.22			
	1572	3316		3316					NA	6.52			
13.4	3934	3568	3934	3568	3751	7%	4981	31%	8.25	7.23	11.10	37%	83%
	7157	6780	7157	6780	6969	4%			16.70	15.76			
	4228	4219	4228	4219	4224	0%			9.06	9.04			
5.5	5184	4421	5184	4421	4803	11%	4765	13%	11.64	9.59	10.52	16%	191%
	5310	4858	5310	4858	5084	6%			11.97	10.77			
	3717	5098	3717	5098	4408	22%			7.64	11.41			
6.89	4361	5771	4361	5771	5066	20%	3959	26%	9.42	13.18	8.32	34%	121%
	3423	3025	3423	3025	3224	9%			6.82	5.69			
	4050	3121	4050	3121	3586	18%			8.57	5.96			
7.79	9	1209	DARK				3611	15%	NA	NA	7.35	21%	94%

	2984	3309	2984	3309	3147	7%			5.57	6.50			
	3967	4183	3967	4183	4075	4%			8.34	8.94			
6.66	3145	2757	3145	2757	2951	9%	3206	15%	6.03	4.93	6.21	22%	93%
	3727	3692	3727	3692	3710	1%			7.67	7.57			
	2711	13	2711	DARK					4.80	NA			
8.14	5177	4244	5177	4244	4711	14%	5050	11%	11.62	9.10	11.29	13%	139%
	5774	5040	5774	5040	5407	10%			13.19	11.26			
	5017	2006	5017						11.20	NA			
7.34	4116	4235	4116	4235	4176	2%	4217	2%	8.75	9.08	9.03	3%	123%
	10	1965	DARK						NA	NA			
	4299	0	4299	DARK					9.25	NA			
8.82	7603	3085		3085			4783	28%	NA	5.86	10.57	34%	120%
	5009	4085	5009	4085	4547	14%			11.17	8.67			
	6687	5049	6687	5049	5868	20%			15.52	11.28			
13	7376	5949	7376	5949	6663	15%	6459	10%	17.24	13.64	14.95	11%	115%
	5922	6897	5922	6897	6410	11%			13.57	16.05			
	6150	2333	6150						14.16	NA			
9.14	2132	3525		3525			3931	14%	NA	7.11	8.24	19%	90%
	4752	3694	4752	3694	4223	18%			10.49	7.58			
	5985	3751		3751					NA	7.74			
6.78	3020	2458	3020	2458	2739	15%	3045	12%	5.68	4.07	5.75	18%	85%
	2929	3031	2929	3031	2980	2%			5.42	5.71			
	3376	3457	3376	3457	3417	2%			6.69	6.91			
11	7	1024	DARK				5255	30%	NA	NA	11.83	35%	108%
	7032	6050	7032	6050	6541	11%			16.39	13.90			
	4327	3609	4327	3609	3968	13%			9.33	7.34			
15.5	6269	7635	6269	7635	6952	14%	7776	24%	14.46	17.87	18.22	25%	118%
	8320	11270	8320	11270	9795	21%			19.53	26.32			
	7016	6143	7016	6143	6580	9%			16.35	14.14			
13.6	6111	6610	6111	6610	6361	6%	6564	25%	14.06	15.33	15.21	27%	112%
	5305	4444	5305	4444	4875	12%			11.96	9.65			
	8132	8780	8132	8780	8456	5%			19.08	20.62			
21	13874	15235	13874	15235	14555	7%	12427	30%	31.93	34.75	28.85	29%	137%
			DARK	DARK					NA	NA			

	5479	8172		8172					NA	19.18			
16.3	8196	7495	8196	7495	7846	6%	7145	10%	19.23	17.53	16.67	11%	102%
	7011	6670	7011	6670	6841	4%			16.34	15.48			
	7401	6094	7401	6094	6748	14%			17.30	14.02			
5.6	2393	2146	2393	2146	2270	8%	3056	24%	3.89	3.19	5.78	36%	103%
	3439	3775	3439	3775	3607	7%			6.86	7.81			
	3525	4961	3525						7.11	NA			
6.24	2350	3178	2350	3178	2764	21%	2997	19%	3.76	6.13	5.61	29%	90%
	3904	2433	3904	2433	3169	33%			8.17	4.00			
	3100	3014	3100	3014	3057	2%			5.90	5.66			
8.45	3811	4122	3811	4122	3967	6%	3239	25%	7.91	8.77	6.30	36%	75%
	4848	3442		3442					NA	6.87			
	2345	2477	2345	2477	2411	4%			3.75	4.13			

Table 22: EDTA v. Li Heparin v. Serum

Summary							
	CLIA	Theranos Reported			Theranos Corrected		
Sample ID	Reported	EDTA	Li Heparin	Serum	EDTA	Li Heparin	Serum
Patient 1	7.28	8.30	6.31	7.38	9.15	6.96	8.14
Patient 2	13.4	14.30	15.65	11.10	15.74	17.22	12.22
Patient 3	5.5	10.49	8.47	10.52	11.55	9.34	11.58
Patient 4	6.89	7.07	4.32	8.32	7.79	4.77	9.16
Patient 5	7.79	9.01	7.76	7.35	9.93	8.55	8.10
Patient 6	6.66	7.63	7.98	6.21	8.41	8.79	6.84
Patient 7	8.14	12.18	16.10	11.29	13.41	17.71	12.42
Patient 8	7.34	5.45	6.97	9.03	6.01	7.69	9.94
Patient 9	8.82	9.78	10.78	10.57	10.76	11.87	11.64
Patient 10	13	12.19	13.35	14.95	13.41	14.69	16.45
Patient 11	9.14	8.01	5.85	8.24	8.82	6.45	9.08
Patient 12	6.78	4.90	6.50	5.75	5.41	7.17	6.34
Patient 13	11	11.66	9.20	11.83	12.84	10.13	13.02
Patient 14	15.5	15.09	15.72	18.22	16.61	17.30	20.04
Patient 15	13.6	17.04	16.61	15.21	18.74	18.27	16.74

Patient 16	21	25.63	18.11	28.85	28.18	19.92	31.72
Patient 17	16.3	22.03	18.51	16.67	24.23	20.36	18.34
Patient 18	5.6	5.28	3.69	5.78	5.83	4.08	6.37
Patient 19	6.24	5.44	7.13	5.61	6.00	7.86	6.19
Patient 20	8.45	6.35	5.30	6.30	7.00	5.85	6.95

Figure 9: EDTA Plasma v. Lithium Heparin Plasma

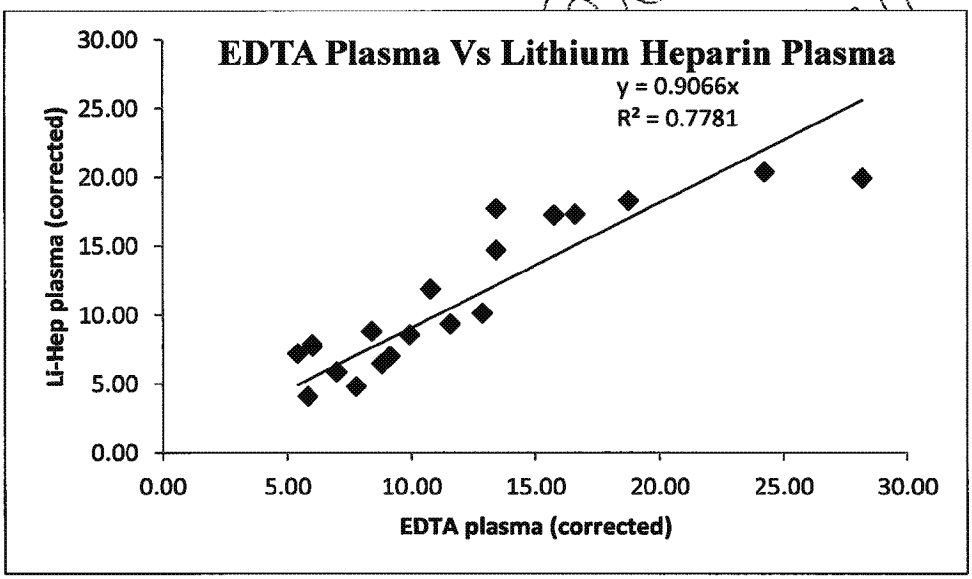
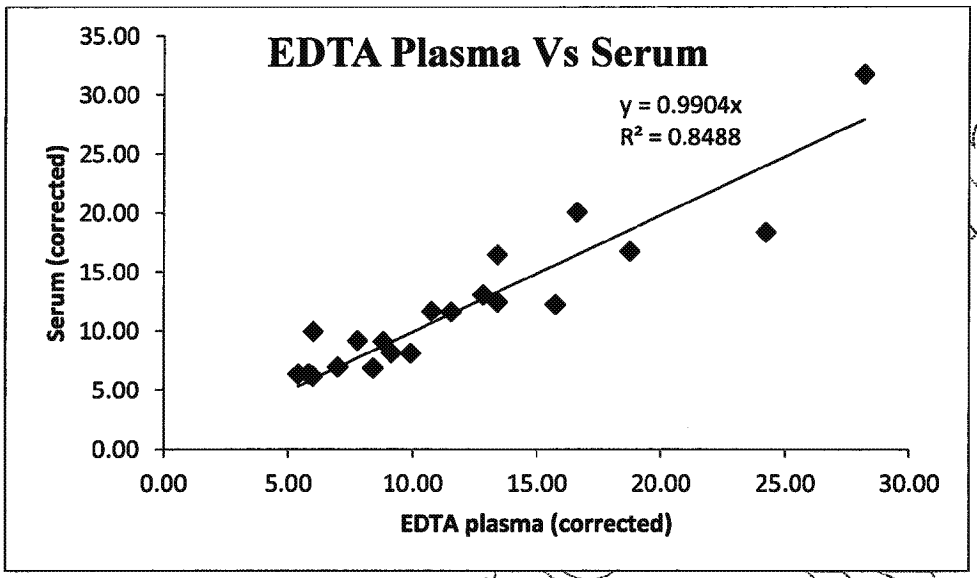


Figure 10: EDTA Plasma v Serum



11 INTERFERENCE

The recovery of analyte spiked into hemolyzed (400mg/dL Hemoglobin), hemolyzed (200mg/dL Hemoglobin), icteric (20mg/dL Bilirubin), and lipemic (3000mg/dL Triglycerides) serum samples were evaluated on the Theranos System and compared to predicate method values. Hemolyzed samples at 400mg/dL had high recoveries at lower levels of prolactin, when the samples were tested at 200mg/dL, they had excellent recovery within 100 ± 20% range. Icteric samples had excellent recovery within 100 ± 20% ranges except for 1 level which had a lightly higher recovery. Lipemic samples had excellent recovery within 100 ± 20% range.

Table 23: Hemolyzed Samples (400mg/dL)

Reported Value [ng/ml]	All Tips		Dark Exclusion		Intra-Cart.		Inter-Cart.		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
495.2	360926	391840	360926	391840	376383	6%	362302	13%	478.55	515.78	480.20	12%	97%
	286695	328097	286695	328097	307396	10%			390.02	439.28			
	407258	398995	407258	398995	403127	1%			534.46	524.44			
187.2	188504	171143	188504	171143	179824	7%	155790	15%	273.05	252.08	233.37	12%	125%
	140170	139886	140170	139886	140028	0%			214.12	213.77			
	167303	127731	167303	127731	147517	19%			247.41	198.61			

12.2	8399	4609	8399				6965	15%	19.72	NA	16.22	16%	133%
	3005	7121		7121					NA	16.61			
	6005	6334	6005	6334	6170	4%			13.79	14.63			
1.44	1324	1960	1324	1960	1642	27%	1747	13%	OORL	2.66	2.07	10%	144%
	1799	1747	1799	1747	1773	2%			2.22	2.07			
	1827	1824	1827	1824	1826	0%			2.29	2.29			

Table 24: Hemolyzed Samples (200mg/dL)

Reported Value [ng/ml]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
338			DARK	DARK			190775	8%	NA	NA	275.78	7%	82%
	187473	205908	187473	205908	196691	7%			271.81	293.93			
	171305	198414	171305	198414	184860	10%			252.28	284.96			
242	100116	112844	100116	112844	106480	8%	126266	19%	163.34	179.76	196.77	16%	81%
	105930	128043	105930	128043	116987	13%			170.88	199.00			
	155642	155021	155642	155021	155332	0%			233.19	232.43			
13.2	5992	6450	5992	6450	6221	5%	5621	12%	13.75	14.92	12.79	14%	97%
	4509	5143	4509	5143	4826	9%			9.83	11.53			
	5869	5765	5869	5765	5817	1%			13.44	13.17			
1.47	1358	1748	1358	1748	1553	18%	1433	22%	1.06	2.08	1.25	38%	85%
	1624	1742	1624	1742	1683	5%			1.74	2.06			
	1023	1100	1023	1100	1062	5%			OORL	OORL			

Table 25: Icteric Samples (20mg/dL)

Reported Value [ng/ml]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
251	142721	172300	142721	172300	157511	13%	172181	12%	217.28	253.48	253.34	10%	101%
	254319	288508							NA	NA			
	192117	181587	192117	181587	186852	4%			277.40	264.72			
131	57348	50190	57348	50190	53769	9%	58918	10%	104.83	94.24	107.11	8%	82%
	58797	56348	58797	56348	57573	3%			106.93	103.37			

	64543	66281	64543	66281	65412	2%			115.17	117.63			
26.7	14152	14504	14152	14504	14328	2%	14950	10%	32.51	33.24	34.17	9%	128%
	21337	16633		16633					NA	37.58			
	13209	16254	13209	16254	14732	15%			30.52	36.82			
4.97	3503	3035	3503	3035	3269	10%	3168	16%	7.04	5.72	6.10	23%	123%
	2372	3535	2372	3535	2954	28%			3.83	7.13			
	3690	2871	3690	2871	3281	18%			7.57	5.25			

Table 26: Lipemic Samples (3000mg/dL)

Reported Value [ng/ml]	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Concentration				
	Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
275	161575	177002	161575	177002	169289	6%	164430	19%	240.44	259.18	243.92	16%	89%
	187034	204556	187034	204556	195795	6%			271.28	292.32			
	119751	136663	119751	136663	128207	9%			188.55	209.77			
57.7	17826	21641	17826	21641	19734	14%	20950	15%	39.94	47.23	45.94	13%	80%
	18414	10725	18414						41.09	NA			
	21328	25543	21328	25543	23436	13%			46.64	54.33			
24.5	9067	12161	9067	12161	10614	21%	11757	17%	21.30	28.27	27.39	16%	112%
	13704	12094	13704	12094	12899	9%			31.57	28.13			
	7754	7531							NA	NA			
4.45	2090	2117	2090	2117	2104	1%	2471	14%	3.03	3.10	4.11	24%	92%
	2646	2788	2646	2788	2717	4%			4.61	5.02			
	3363	2716		2716					NA	4.81			

12 CROSSREACTIVITY

Information about cross-reacting analytes from predicate method insert.

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

12.1.1 A statistical summary table showing the mean, and CV of each concentration level

12.1.2 Theranos calculated concentration (all samples should be "OORL")

12.1.3 % cross-reactivity where applicable

The Theranos Prolactin assay does not show any cross reactivity with FSH, Human Placental Lactogen (hPL), and LH. There was some cross relativity at very high level of TSH (50mIU/ml), but no cross reactivity at lower levels of TSH (25 and 10mIU/ml).

Table 27: Cross-reactive Samples

Sample ID	Reported Value [mIU/ml]	All Tips		Dark Exclusion		Intra-Cart.		Inter-Cart.		Concentration				
		Tip1	Tip2	Tip1	Tip2	Mean	%CV	Mean	%CV	Tip 1	Tip 2	Mean	%CV	%Recovery
FSH (mIU/ml)	300	609	625	609	625	617	2%	630	3%	OORL	OORL	OORL	OORL	OORL
		1962	1038							NA	NA			
		642	645	642	645	644	0%			OORL	OORL			
HCG (mIU/ml)	500	489	507	489	507	498	3%	570	15%	OORL	OORL	OORL	OORL	OORL
		697	542	697	542	620	18%			OORL	OORL			
		617	1638	617						OORL	NA			
hPL (ug/ml)	200	1163	1534	1163	1534	1349	19%	1256	13%	OORL	1.51	OORL	OORL	OORL
		1203	1365	1203	1365	1284	9%			OORL	1.08			
		1184	1085	1184	1085	1135	6%			OORL	OORL			
LH (ng/ml)	40	2311	690		690			685	13%	NA	OORL	OORL	OORL	OORL
		807	686	807	686	747	11%			OORL	OORL			
		682	560	682	560	621	14%			OORL	OORL			
TSH (mIU/ml)	50	1503	1769	1503	1769	1636	11%	1553	22%	1.43	2.13	1.56	33%	3%
		1155	1142	1155	1142	1149	1%			OORL	OORL			
		1784	1962	1784	1962	1873	7%			2.18	2.67			
TSH (mIU/ml)	25	970	922	970	922	946	4%	1244	21%	OORL	OORL	OORL	OORL	OORL
		1183	1431	1183	1431	1307	13%			OORL	1.24			
		1481	1475	1481	1475	1478	0%			1.37	1.36			
TSH (mIU/ml)	10	1333	1186	1333	1186	1260	8%	1285	9%	OORL	OORL	OORL	OORL	OORL
		1633	1461		1461					NA	1.32			
		1170	1277	1170	1277	1224	6%			OORL	OORL			

13 SAMPLE STABILITY

The stability of analyte (stored in screw cap cyro vials) 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 indicate that the samples can be processed up to 72h if stored at 4 degrees and room temperature.

Table 28: Analyte Stability Summary

	4C						Room Temperature					
Level	0hr	1hr	4hr	12hr	24hr	72hr	0hr	1hr	4hr	12hr	24hr	72hr
637	492.96	561.59	450.52	638.06	585.27	525.42	597.21	611.15	558.32	458.41	668.08	532.45
70.4	70.71	82.86	63.63	74.47	58.86	70.71	65.13	57.93	59.84	72.29	63.24	57.36
0	1.65	OORL	OORL	1.23	OORL	OORL	1.18	OORL	3.61	1.03	OORL	1.41

Figure 11: Analyte Stability at 4C

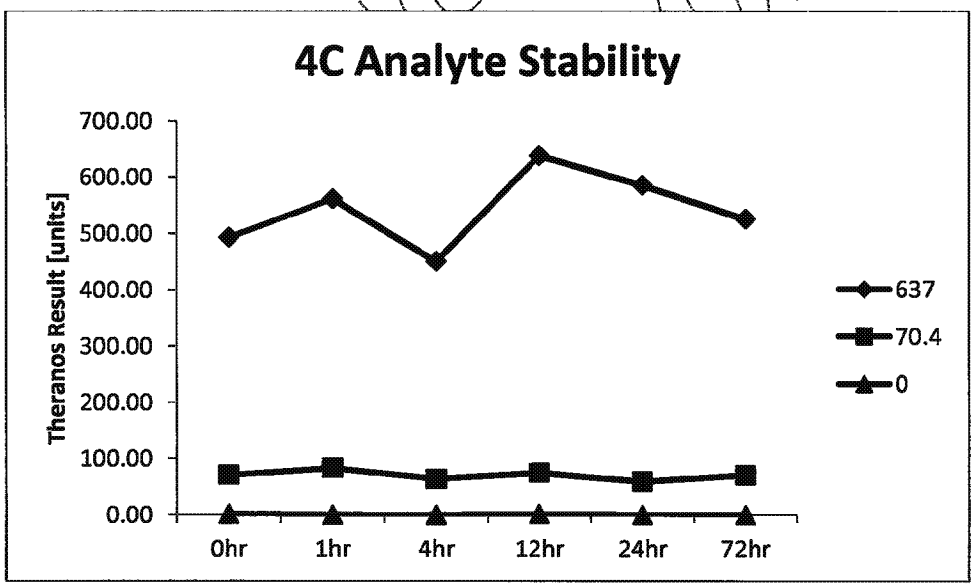
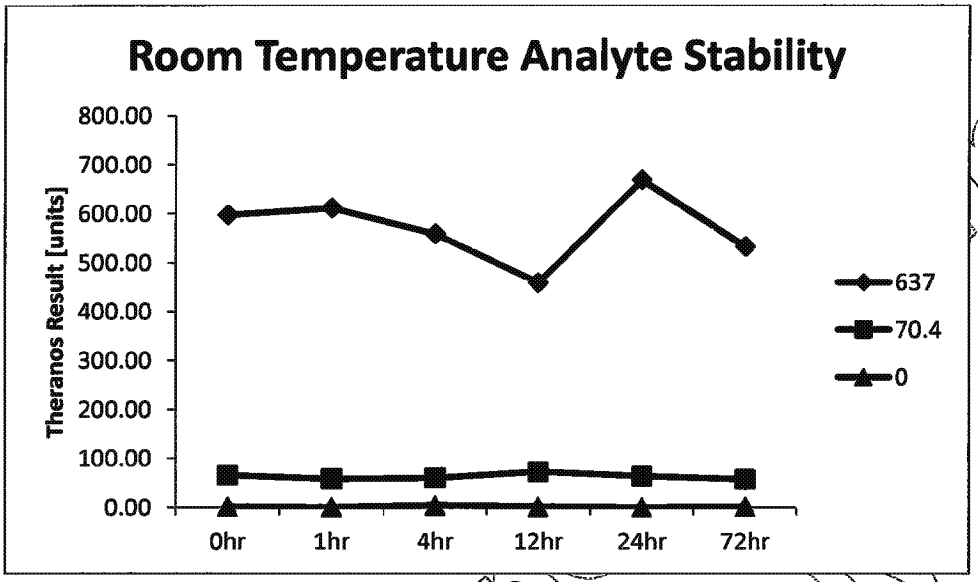


Figure 12: Analyte Stability at Room Temperature



14 Reagent Stability

Theranos System reagents are stable up to 7 weeks at 4C

Figure 13: Capture Antibody Stability at Room temperature and 4C

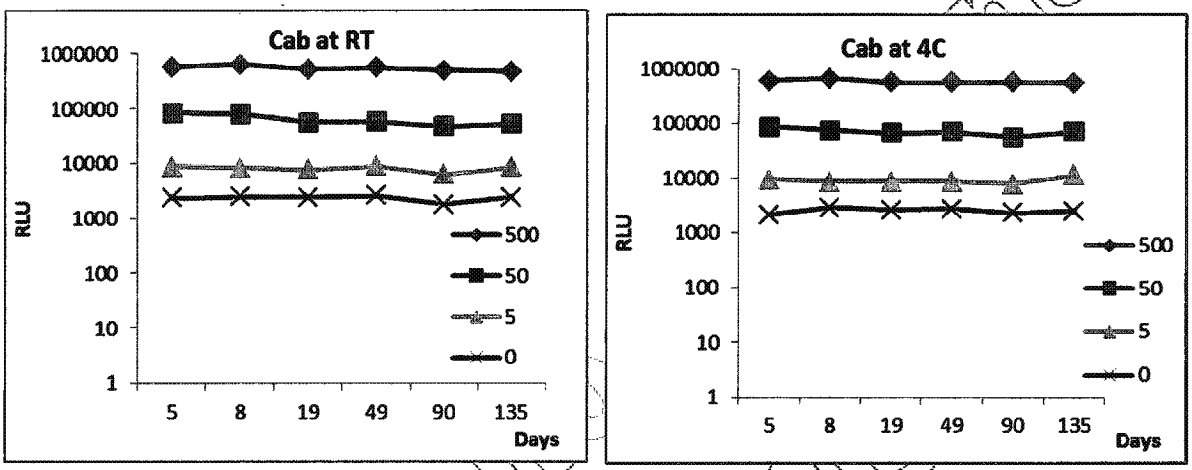
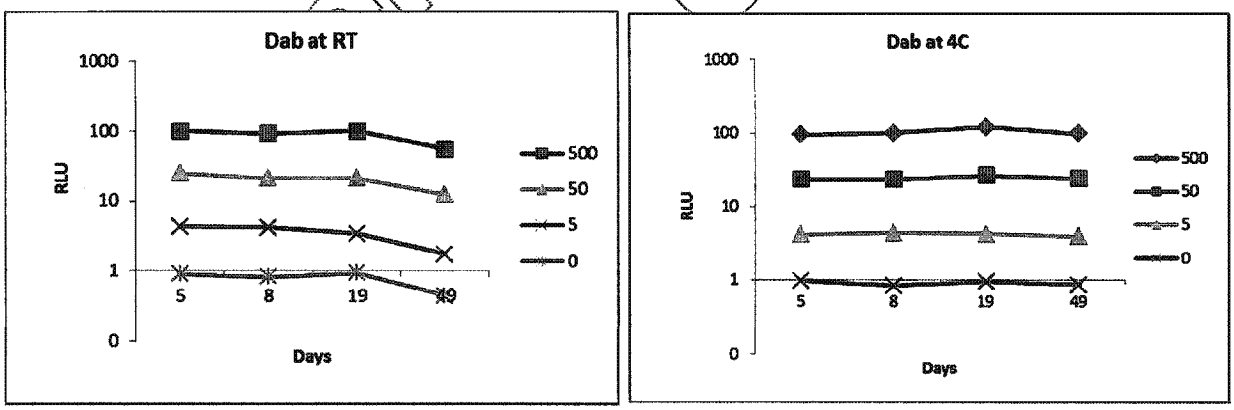


Figure 14: Detection Antibody Stability at Room temperature and 4C



15 REFERENCES

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