
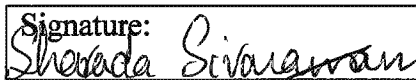


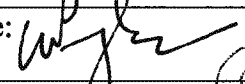
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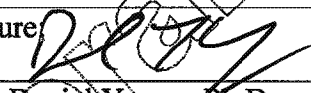
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1 ASSAY BACKGROUND

17 Beta-Estradiol is a steroid hormone, the predominant sex steroid in human females, and also present in males. It is one of three estrogens including estrone (E1) estradiol (E2), and estriol (E3). Estriol levels are high only during pregnancy, while estrone levels are low during reproductive years and rise after menopause. In females of reproductive age with normal ovaries, estradiol production during the menstrual cycle peaks the 1-2 days before ovulation and then falls as LH levels peak.

In the blood, estradiol is bound with high affinity to Steroid Hormone Binding Globulin (SHBG) a homodimeric glycoprotein with an internal hydrophobic domain. Human SHBG binds testosterone and its metabolites with highest affinity, followed closely by affinity for estradiol. Human SHBG differs from many other mammalian SHBGs in the fact that it binds estradiol with such high affinity. Estradiol also associates with low affinity to other plasma proteins such as Human Serum Albumin (HSA).

Since most of the estradiol in blood is bound to these proteins, many antibodies raised against estradiol haptens are not able to bind to the appropriate epitope in a biological matrix. The potential unavailability of SHBG-bound estradiol presents a challenge for assay design, and two main approaches to the problem can be used. One approach is to attempt to displace the estradiol from SHBG using a steroid with higher binding affinity such as Dihydrotestosterone (DHT). However in multiplexing assays this presents many challenges and limitations on the combination of analytes to be measured. Another approach is to create an antibody that is able to bind to estradiol even in its SHBG-bound state, likely a polyclonal antibody.

In post menopausal women, a high estradiol concentration is predictive of preserved bone mass and reduced fracture rates. Estradiol reduces bone resorption by inducing apoptosis of osteoclasts and is therefore protective against osteoporosis. Estradiol may also assist in attenuating the progression of diabetic kidney disease primarily by acting on TGF-Beta-1.

1.1 Theranos System Specification

This assay is designed to detect Estradiol in human EDTA plasma and Serum. Li-heparin plasma is not recommended to be used as a sample for the Theranos Estradiol assay. The assay has a reportable range of 25 – 1200 pg/ml (calibrator lot specific: current lot 26.9 – 1293 pg/ml). The calibrator values are verified by reference method. No NIBSC WHO material is available for Estradiol. Samples are stable at 4C or RT for 72 hours. If samples are not analyzed within 72 hours of collection, samples should be stored at -20C or lower.

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Table 1 Theranos Estradiol Assay Specification

Assay	Theranos Estradiol Assay
Reportable Range	Nominal value 25 - 1200 pg/ml (validation batch: 26.9 - 1293 pg/ml)
Accepted sample type	EDTA plasma and Serum
Sample Stability	Up to 72 hours at 4 degree Celsius or Room Temperature

1.2 Reference Assay

The SIEMENS Immulite 2000 Estradiol test was used as a predicate method. The reportable range is 20-2000 pg/ml. Hemolysis (packed red blood cells in concentration up to 30 µl/ml), lipemia (up to 3000 mg/dL triglycerides), and icterus (up to 200 mg/L bilirubin), does 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 $100 \pm 20\%$ ($100 \pm 25\%$ at LLOQ and ULOQ standards) of their nominal values, and a minimum of six unique standard concentrations must be within the assay range.

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3.2 Calibrator preparation

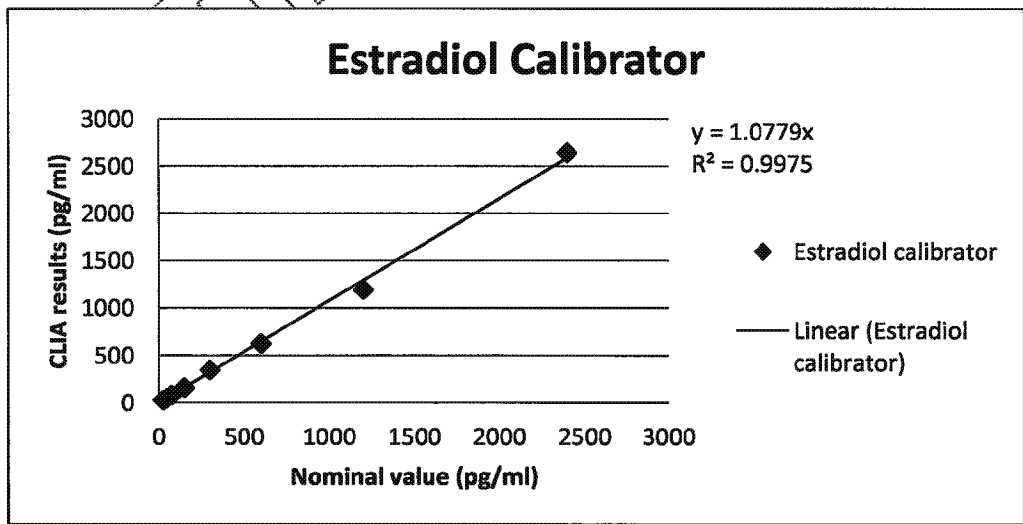
Estradiol assay calibrators were prepared by series diluting Estradiol standard material into charcoal adsorbed steroid depleted serum. Each calibrator was analyzed by predicate method. A graph was plotted (Nominal vs CLIA values) and the reassigned value was calculated from the slope.

$$\text{Reassigned Value (pg/mL)} = \text{Nominal Value} * 1.0779$$

Table 2 Calibrator preparation

Estradiol	nominal conc (pg/ml)	CLIA results (pg/ml)	Reassigned Value (pg/ml)
Calibrator 1	2400	2641	2587.0
Calibrator 2	1200	1193	1293.5
Calibrator 3	600	623	646.7
Calibrator 4	300	344	323.4
Calibrator 5	150	157	161.7
Calibrator 6	75	77.3	80.8
Calibrator 7	50	48.2	53.9
Calibrator 8	25	23.8	26.9
Calibrator 9	12.5	<20	13.5
Calibrator 10	0	<20	0.0

Figure 1 Calibrator re-assignment



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Calibration (Edison 3.5) For All Validation Experiments

Two sets of device were calibrated. Each set of device was calibrated by nozzle (tip). For both sets, precision and accuracy were within $100 \pm 25\%$ 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 calibrated individually. Each level had 15 runs on each device. All three levels were run on each device.

4.3 Precision summary

Table 3: Acceptable Performance

Decision Level	Acceptable performance	TAE(%)	Precision (%)	Allowable Bias (%)
60 pg/ml	25%	25	21.1	3.9
450 pg/ml	24%	24	20.1	3.9

Table 4: Precision summary

Level	Assigned value (pg/ml)	Mean Calculated	
		Concentration (pg/ml)	%CV
1	550	554	20.1%
2	183.3	174.4	20.8%
3	73.3	65.5	21.1%


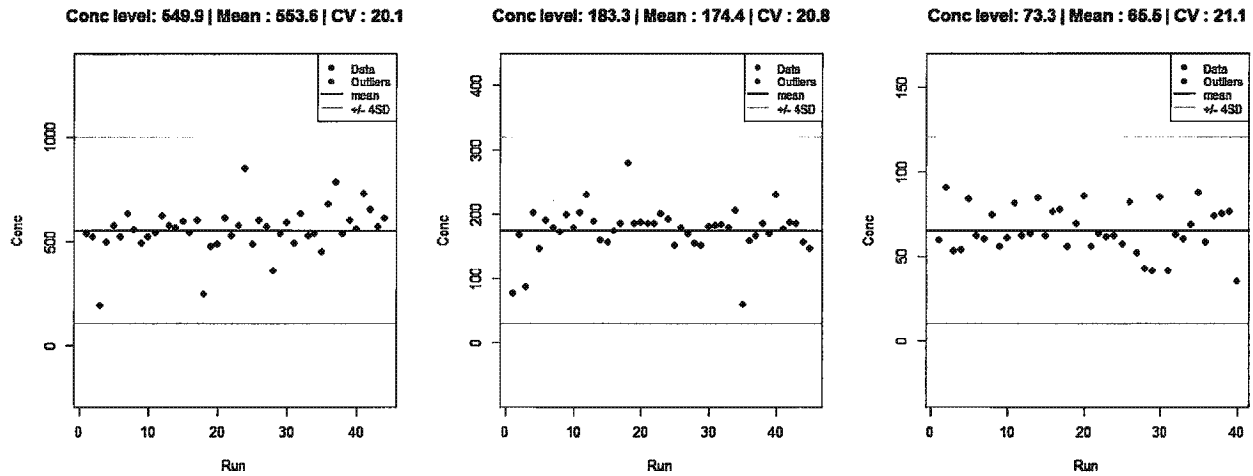
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Figure 2: Precision Plots

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



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5 ACCURACY/COMPARABILITY

- 5.1 To test the accuracy of the assay on the Theranos System, 145 unique patient samples were assayed using both the predicate method (Siemens Immulite) and the Theranos System. Of these samples 47 were within the medical decision range (150 - 500 pg/ml), 50 were below 150pg/ml, and 48 were higher than 500pg/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: Weighted Deming fitting was chosen for Estradiol method comparison. Equation between Theranos results and Immulite results was determined as $y=0.98x+19.34$

Table 5: Method comparison summary

Sample ID	CLIA value (pg/ml)	Theranos results (pg/ml)	Theranos Bias-corrected results (pg/ml)
Patient 1	< 20.0	OORL	OORL
Patient 2	27.7	OORL	OORL
Patient 3	< 20.0	OORL	OORL
Patient 4	< 20.0	OORL	OORL
Patient 5	< 20.0	OORL	OORL
Patient 6	28.8	OORL	OORL
Patient 7	57.3	56.8	38.3
Patient 8	71.4	66.8	48.4
Patient 9	30.9	22.2	3.0
Patient 10	27.4	OORL	OORL
Patient 11	51.7	93.8	76.0
Patient 12	< 20.0	OORL	OORL
Patient 13	< 20.0	33.8	14.8
Patient 14	< 20.0	OORL	OORL
Patient 15	53.4	39.9	21.0
Patient 16	47.5	OORL	OORL
Patient 17	76.3	227.5	212.4
Patient 18	27.1	497.3	487.7
Patient 19	25.8	55.4	36.8

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Patient 20	87.8	91.7	73.9
Patient 21	38.5	69.8	51.5
Patient 22	29.8	21.7	2.4
Patient 23	< 20.0	OORL	OORL
Patient 24	< 20.0	34.7	OORL
Patient 25	42.5	OORL	OORL
Patient 26	610	658	652
Patient 27	1273	1233	1239
Patient 28	576	562	554
Patient 29	1805	2365	2394
Patient 30	1101	988	989
Patient 31	1910	1735	1751
Patient 32	3261	2070	2093
Patient 33	2503	1326	1334
Patient 34	994	813	810
Patient 35	1693	1050	1051
Patient 36	456	669	663
Patient 37	1431	1954	1974
Patient 38	1941	823	820
Patient 39	737	1192	1196
Patient 40	981	1947	1967
Patient 41	1627	2126	2149
Patient 42	494	551	543
Patient 43	1299	1243	1249
Patient 44	1318	1178	1183
Patient 45	1604	1130	1134
Patient 46	2625	2095	2118
Patient 47	520	538	529
Patient 48	859	602	595
Patient 49	1145	1014	1015
Patient 50	418	255	240
Patient 51	>2000	2048	2070
Patient 52	550	623	616
Patient 53	1214	1512	1523
Patient 54	1461	1362	1370
Patient 55	1130	1469	1479

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Patient 56	1657	2435	2465
Patient 57	1721	1592	1605
Patient 58	1514	1514	1526
Patient 59	1195	1169	1173
Patient 60	1161	1539	1551
Patient 61	961	594	586
Patient 62	>2000	2677	2712
Patient 63	350	436	426
Patient 64	850	709	704
Patient 65	1325	1256	1262
Patient 66	1024	1109	1112
Patient 67	918	1163	1167
Patient 68	1263	2764	2801
Patient 69	1784	1431	1440
Patient 70	2333	2451	2481
Patient 71	1753	1835	1852
Patient 72	1259	1237	1242
Patient 73	609	394	382
Patient 74	2401	1933	1953
Patient 75	942	819	816
Patient 76	39.8	OORL	OORL
Patient 77	49.8	102	84.6
Patient 78	32.4	OORL	OORL
Patient 79	23.9	OORL	OORL
Patient 80	20.9	OORL	OORL
Patient 81	22.4	OORL	OORL
Patient 82	56.2	63.1	44.7
Patient 83	25	OORL	OORL
Patient 84	45.9	OORL	OORL
Patient 85	149	283	269
Patient 86	176	161	145
Patient 87	36.2	OORL	OORL
Patient 88	32.1	56.1	37.5
Patient 89	117	202	186
Patient 90	37.5	OORL	OORL
Patient 91	99.2	73.7	55.5

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Patient 92	62.3	36.6	17.6
Patient 93	87.7	55.4	36.8
Patient 94	34.9	67.0	48.6
Patient 95	38.6	34.3	15.3
Patient 96	22.8	OORL	OORL
Patient 97	121	219	204
Patient 98	30.7	OORL	OORL
Patient 99	159	51.5	32.8
Patient 100	36.7	41.1	22.2
Patient 101	199	195	179
Patient 102	279	292	278
Patient 103	223	336	323
Patient 104	478	572	564
Patient 105	386	572	564
Patient 106	383	247	233
Patient 107	372	461	451
Patient 108	368	333	320
Patient 109	152	232	217
Patient 110	340	339	326
Patient 111	249	196	181
Patient 112	246	367	355
Patient 113	266	247	233
Patient 114	212	119	102
Patient 115	176	104	86.3
Patient 116	181	66.4	48.0
Patient 117	432	349	336
Patient 118	356	1093	1096
Patient 119	187	120	103
Patient 120	295	306	292
Patient 121	272	255	240
Patient 122	246	192	176
Patient 123	223	325	312
Patient 124	295	365	353
Patient 125	351	349	337
Patient 126	208	241	226
Patient 127	333	598	590

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Patient 128	182	127	110
Patient 129	253	367	355
Patient 130	292	185	169
Patient 131	310	248	233
Patient 132	406	600	592
Patient 133	248	399	387
Patient 134	206	212	197
Patient 135	275	722	717
Patient 136	184	338	325
Patient 137	204	344	331
Patient 138	224	200	184
Patient 139	315	359	346
Patient 140	176	147	131
Patient 141	147	226	211
Patient 142	149	115	97.3
Patient 143	1910	1419	1429
Patient 144	1472	1481	1491
Patient 145	1973	OORH	OORH

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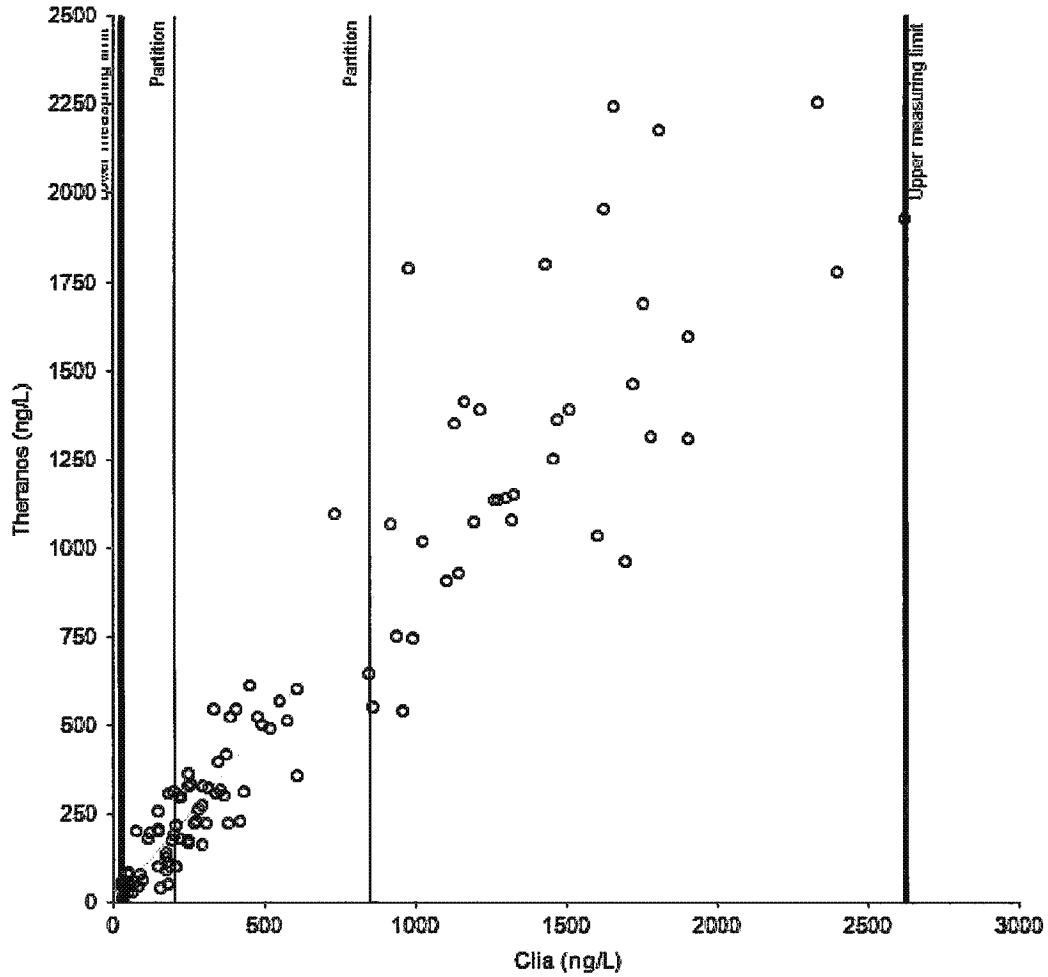
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Figure 3: Scatter Plot – CLSI Guideline EP09-A2-IR Section 4.2

Scatter plot

CLSI guideline EP09-A2-IR section 4.2

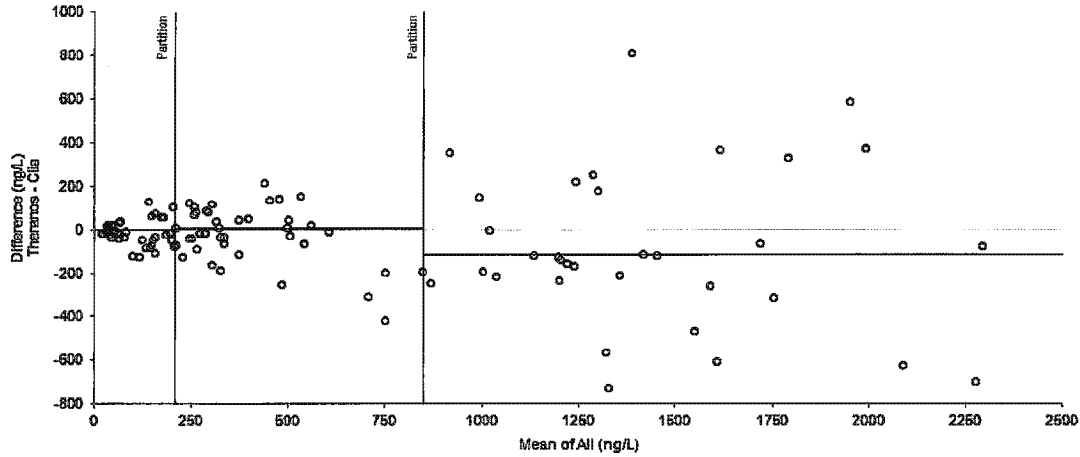


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Figure 4: Difference Plot – CLSI Guideline EP09-A2-IR Section 4.2

Difference plot

CLSI guideline EP09-A2-IR section 4.2



Adequate range test

CLSI guideline EP09-A2-IR section 4.5


$r = 0.938$

$r < 0.975$ 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
< 208	36	-0.92220938	62.09771284
≥ 208 and < 850	38	1.440454955	114.4950934
≥ 850	36	-120.479578	348.1226843

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Comparability

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
1	60.00000000	-0.92220938	10.34981880	333052577 to 20.088	2.400000000
2	450.00000000	1.440454955	18.57355636	193145802 to 39.074	18.000000000

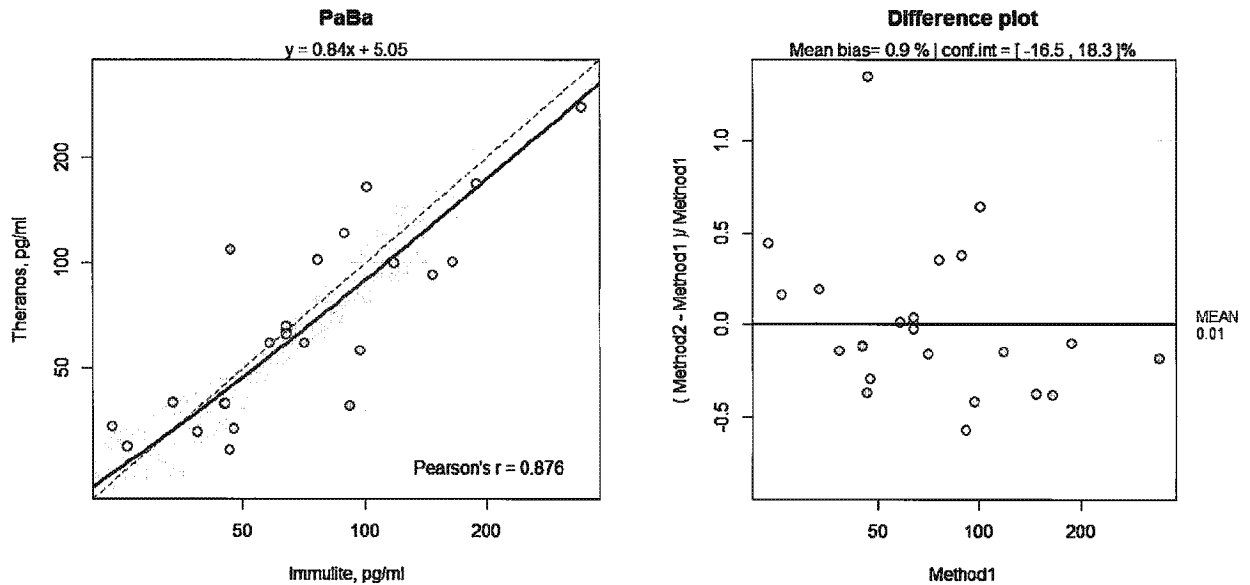
Difference is less than allowable bias: 4%.

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 measurements, and then compare the mean bias between the two methods with the total allowable bias.

$$\begin{aligned}
\text{Total allowable bias} &= \text{Total allowable error} - \text{Avg Imprecision} \\
&= 24\% - 20.2\% \\
&= 3.8\%
\end{aligned}$$

As seen in figure, after correction, the mean bias is 0.9%, with a 95% confidence interval around it of [-16.5%, 18.3%]. This bias is within the total allowable bias, thus validating the bias correction.

Figure 5: Verification of Bias Correction



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Table 6: Verification of Bias Correction

SampleID	Immulite	Theranos
Patient 1	88.5	122.1
Patient 3	44.9	39.8
Patient 6	91.3	39.3
Patient 7	38.5	33.0
Patient 8	63.7	64.2
Patient 9	46.6	109.7
Patient 10	100.7	165.5
Patient 13	63.8	66.1
Patient 14	96.4	56.4
Patient 16	58.2	59.1
Patient 17	342.0	279.0
Patient 18	25.8	30.0
Patient 20	75.9	102.8
Patient 22	117.5	100.2
Patient 23	164.0	101.2
Patient 25	33.6	40.0
Patient 26	70.4	59.2
Patient 27	45.0	39.8
Patient 28	187.5	168.8
Patient 30	47.4	33.6
Patient 31	46.2	29.3
Patient 33	147.0	92.4
Patient 35	23.7	34.2
Patient 37	63.9	62.6

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6 DILUTION LINEARITY

- 6.1 A high analyte clinical sample was selected and serially diluted with a low analyte clinical sample to a total of 8 points to test the 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 $100 \pm 20\%$ of their nominal value, and when plotted, the R^2 value should be equal or greater than 0.95.

Table 7: Dilution Linearity

CLIA Conc (pg/ml)	SampleID	RLU						Theranos measured concentration (pg/ml)						result
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
2333	High Patient Sample	3218	2348	2490	2082	2077	2633	OORH	1202.603	OORH	OORH	OORH	OORH	OORH
1092	Dilution 1	3934	5263	3684	5354	4407	3911	992	625	1133	641	1044	1011	1051
531	Dilution 2	6527	6196	5122	5369	5897	6765	390	514	679	636	683	454	553
266	Dilution 3	8896	7133	7942	7906	9971	9484	289	425	362	280	270	266	250
132	Dilution 4	12385	13748	14832	13766	13198	9683	137	124	119	111	143	256	110
69	Dilution 5	15289	15541	15629	dark	14077	13242	90	90	104	INVALID	120	139	91
44.7	Dilution 6	19636	18955	19937	16768	17053	14419	45	50	38	77	66	113	47
29.2	Low Patient Sample	22680	22851	22240	22052	21545	18262	OORL	OORL	OORL	40	OORL	51	OORL

Table 8: Dilution Linearity Summary

Sample ID	Nominal Value [pg/ml]	Theranos Result [pg/ml]	% Recovery
High Patient Sample	2333	OORH	—
1:2	1092	1051	96%
1:4	531	553	104%
1:8	266	250	94%
1:16	132	110	83%
1:32	69	91	133%
1:64	44.7	47	104%
Low Patient Sample	29.2	OORL	—


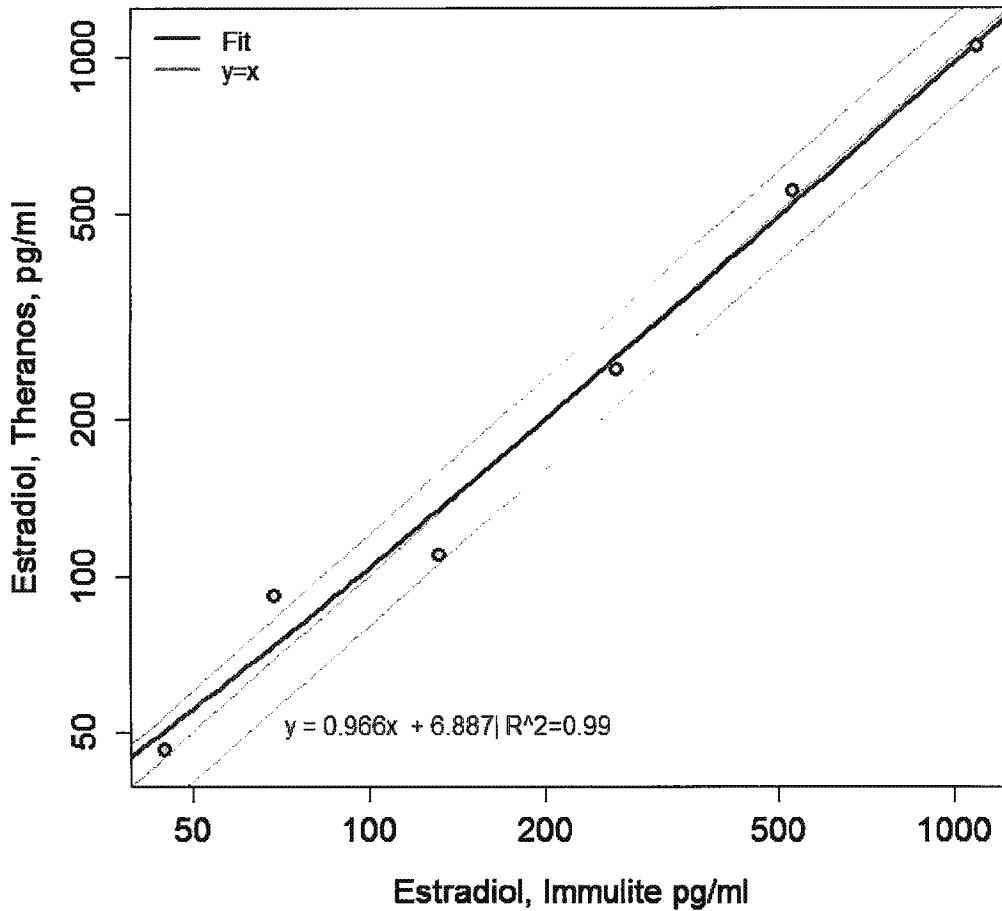
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Figure 6: Dilution Linearity





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7 REFERENCE RANGE

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

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

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

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

The reference interval established from the predicate method is from ND (not detectable) to 400 pg/ml for female donors at different phases in ovulatory cycle.

To verify reference range in Theranos assay, 30 fingerstick samples were collected from 10 unique female donors on three different dates and analyzed by Theranos method. Serum samples from the same donors at the same collection were analyzed by reference method. 29 out of 30 samples had valid results except one sample had a non assay related issue. 28 out of 29 samples had Estradiol value less than 400pg/ml and were within reference range.

Overall, 96.6 % of the patients tested were within the reference range, and is acceptable for verification of the reference range.

Table 9 Summary of reference range verification

Sample ID/Date of collection		Reported Value [pg/mL]	Theranos value
			[pg/mL]
8/18/2014	FS 25	206	356
	FS 26	68.8	59
	FS 27	54.5	INVALID
	FS 28	31.1	99
	FS 29	152	182

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	FS 30	473	489
	FS 31	85.8	119
	FS 32	30.8	257
	FS 33	216	247
	FS 34	67.1	97
8/22/2014	FS 25	45.8	39
	FS 26	29.1	OORL
	FS 27	74.7	78
	FS 28	35	OORL
	FS 29	275	291
	FS 30	434	304
	FS 31	72.2	105
	FS 32	<20.0	OORL
	FS 33	42	98
	FS 34	38.3	98
8/25/2014	FS 25	47.4	72
	FS 26	29.5	OORL
	FS 27	163	206
	FS 28	23.1	OORL
	FS 29	135	148
	FS 30	626	357
	FS 31	86.4	173
	FS 32	24.5	OORL
	FS 33	35.2	130
	FS 34	83	144

8 BLOOD COLLECTION DEVICE (BCD) COMPARISON


To compare EDTA plasma collected from Theranos blood collection device and venipuncture, 40 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.

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Results of fingerstick samples correlated with results of venous samples very well. The slope is 0.93, the R2 of correlation is 0.9.

Table 10: Venous v. Fingerstick results

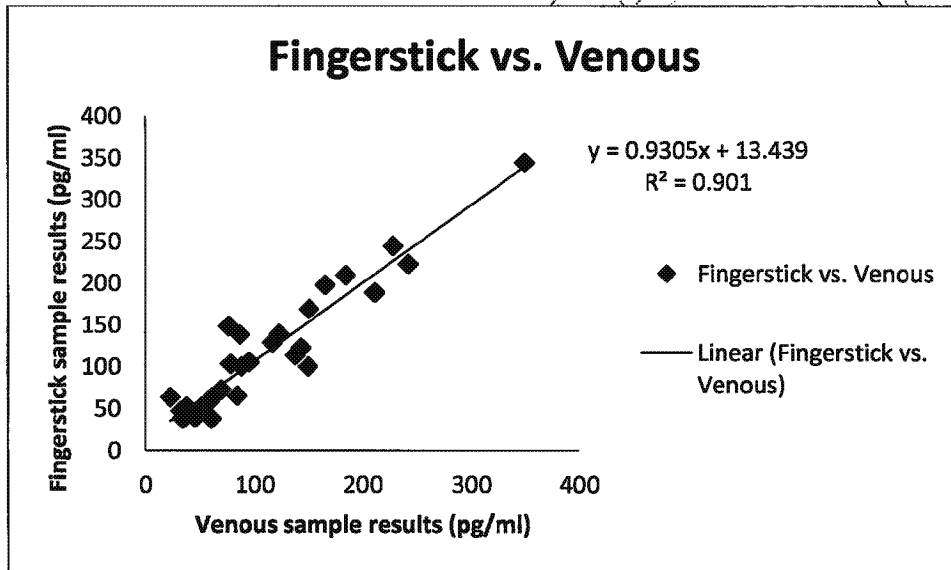
Sample	Estradiol results (pg/ml)	
	Venous	Fingerstick
Patient 1	34.9	38.6
Patient 2	348.9	344.1
Patient 3	OORL	OORL
Patient 4	OORL	OORL
Patient 5	76.0	148.2
Patient 6	184.6	209.0
Patient 7	149.6	100.0
Patient 8	87.9	100.0
Patient 9	84.3	65.1
Patient 10	38.0	51.8
Patient 11	211.3	188.3
Patient 12	61.5	63.2
Patient 13	122.6	139.1
Patient 14	165.6	197.4
Patient 15	51.6	52.1
Patient 16	95.3	105.4
Patient 17	69.4	72.3
Patient 18	45.4	44.8
Patient 19	143.0	122.1
Patient 20	34.5	37.4
Patient 21	78.0	103.1
Patient 22	OORL	OORL
Patient 23	58.9	58.4
Patient 24	35.6	46.9
Patient 25	228.1	244.2
Patient 26	150.6	168.8
Patient 27	OORL	OORL
Patient 28	60.6	38.1

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Patient 29	242.2	222.2
Patient 30	OORL	OORL
Patient 31	116.7	128.7
Patient 32	86.6	138.1
Patient 33	137.6	113.7
Patient 34	32.9	47.1
Patient 35	37.6	45.6
Patient 36	OORL	OORL
Patient 37	OORL	OORL
Patient 38	45.6	39.3
Patient 39	23.1	63.2
Patient 40	OORL	OORL

Internal

Figure 7: Comparison of fingerstick and venous blood samples



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9 ANALYTICAL SENSITIVITY

9.1 Verification of Limit of Blank

9.1.1 60 replicates of blank were measured and calculated from calibration curve.

9.1.2 Results: One out of 60 replicates had quantifiable results.

9.2 Verification of Limit of Quantification

9.2.1 60 replicates of sample at LLOQ level were measured and calculated from calibration curve.

9.2.2 Results: 33 out of 60 replicates had quantifiable results.

9.3 Replicates of 1/2 LLOQ

9.3.1 The LLOQ sample was diluted 1:1 with blank and 20 replicates of the 1/2xLLOQ sample were tested.

9.3.2 Results: 4 out of 20 replicates had quantifiable results.

Table 11: Verification of blank

SampleID	B/L						Measured concentration (pg/ml)						result
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
Blank	46854	44070	49219	47630	43419	34935	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	51178	48766	45401	47327	46501	39375	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	51556	52052	52346	57281	37241	43930	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	38612	40868	40155	40462	38217	46424	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	54789	60286	60288	60209	62284	59548	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	32765	36691	37976	37711	40183	42687	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	34634	38406	35156	39085	41155	38701	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	36109	38889	38252	41256	42486	43474	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	41223	57945	47955	44846	50599	41999	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	44812	39709	36036	39516	38539	32079	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	57443	55749	57932	58998	60688	60821	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	34332	32454	31692	210	210	209	OORL	OORL	OORL	OORH	OORH	OORH	OORL
Blank	59210	55504	58449	31053	59325	52226	OORL	OORL	OORL	34.5	OORL	OORL	OORL
Blank	39533	42545	42164	46410	dark	45988	OORL	OORL	OORL	OORL	INVALID	OORL	OORL



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Blank	40684	36910	41386	44764	45904	39022	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48179	55326	55884	52024	51974	36140	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48694	44626	48161	49928	47966	40681	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	47030	50460	44801	51895	41705	37299	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	38902	40429	43732	41453	42720	34564	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	36826	40536	42740	43509	40319	41147	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	50680	42427	41307	44452	48750	50878	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	52404	56518	51020	56250	63707	55135	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	40177	43764	42821	45793	36600	35639	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48077	45758	36585	48186	42110	34284	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	45511	46489	48889	54374	57776	54300	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	42164	42381	39023	45445	42411	35516	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	25615	28388	35005	37237	dark	36895	35.8	31.0	OORL	OORL	INVALID	OORL	OORL
Blank	52671	49851	49182	47610	45724	39674	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	31417	30230	33776	38887	32712	30060	28.0	27.1	OORL	OORL	OORL	OORL	OORL
Blank	54071	54015	54298	52674	49264	39028	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	33688	34465	34157	34823	37427	33040	OORL	OORL	OORL	29.5	OORL	OORL	OORL
Blank	34025	37039	37076	40244	43331	42877	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	42067	42071	38221	45488	44818	37757	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	51595	49949	62279	59239	64080	62054	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	35854	30626	42742	36725	40115	40517	OORL	OORL	OORL	27.4	OORL	OORL	OORL
Blank	49766	47623	58681	60756	51446	43904	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	40759	44576	47383	48811	47237	43221	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	42020	38906	37845	45836	44786	40502	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	43060	44028	42088	48242	35749	32462	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	40040	36269	32853	40495	42249	46500	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	37594	38914	41616	36000	42213	41128	OORL	OORL	OORL	28.1	OORL	OORL	OORL
Blank	38713	45650	45264	44915	47475	41190	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	35397	32722	dark	33127	30860	31207	OORL	OORL	INVALID	31.6	OORL	OORL	OORL
Blank	41537	42542	41221	44128	47244	45076	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	50818	52009	52183	50212	49924	42227	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48790	45248	39057	48962	dark	49012	OORL	OORL	OORL	OORL	INVALID	OORL	OORL
Blank	45795	45842	46942	49158	46048	40920	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	48911	47464	50214	48217	49156	34353	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	33179	24770	dark	32306	25900	20108	OORL	40.3	INVALID	32.7	OORL	56.3	INVALID
Blank	34975	32464	38312	38162	41636	40379	OORL	OORL	OORL	OORL	OORL	OORL	OORL

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Blank	39238	40332	36866	46734	42028	43028	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	27027	30179	34453	32847	37435	39881	33.5	27.2	OORL	31.9	OORL	OORL	OORL
Blank	38385	40281	38152	41891	46964	45051	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	34991	45348	41397	36800	39794	32826	OORL	OORL	OORL	27.3	OORL	OORL	OORL
Blank	32927	35573	36493	36823	40515	40566	OORL	OORL	OORL	27.3	OORL	OORL	OORL
Blank	29966	33132	31124	36102	36989	37866	29.6	OORL	OORL	28.0	OORL	OORL	OORL
Blank	35168	34219	38975	37988	405	333	OORL	OORL	OORL	OORL	OORH	OORH	OORL
Blank	29369	26747	dark	25028	29184	19540	30.3	34.9	INVALID	46.6	OORL	60.1	43.0
Blank	42291	40906	40003	40447	39618	31097	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Blank	34918	36604	31000	32274	dark	39801	OORL	OORL	27.11056	32.7146	INVALID	OORL	OORL

Table 12: Verification of LLOQ

SampleID	RLU						Measured concentration (pg/ml)						result
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
LLOQ	28231	36305	27523	28267	33482	37804	31.8	OORL	34.2	39.3	OORL	OORL	OORL
LLOQ	26866	30533	31152	18591	33262	36987	33.8	OORL	OORL	71.0	OORL	OORL	OORL
LLOQ	26277	29706	28194	31088	dark	23793	34.7	28.2	32.7	34.5	INVALID	36.3	33.3
LLOQ	22942	25531	24806	30589	dark	29865	41.1	38.1	41.4	35.2	INVALID	OORL	39.0
LLOQ	26318	28735	29594	30808	29355	24521	34.6	30.2	29.7	34.9	OORL	33.1	32.5
LLOQ	32809	27978	27881	29806	30983	33068	OORL	32.0	33.4	36.5	OORL	OORL	OORL
LLOQ	32445	33381	24495	29239	31446	24623	26.9	OORL	42.4	37.5	OORL	32.7	34.9
LLOQ	27883	27424	dark	26325	27121	25762	32.3	33.3	INVALID	43.4	OORL	28.0	34.2
LLOQ	33586	34595	34257	20475	39112	34584	OORL	OORL	OORL	61.9	OORL	OORL	OORL
LLOQ	18222	28842	20402	26365	24301	29440	55.6	30.0	58.4	43.3	34.0	OORL	44.3
LLOQ	16417	15641	16036	22209	dark	21029	64.2	83.3	87.0	55.1	INVALID	50.7	68.1
LLOQ	20828	26045	25322	26278	25374	27279	46.5	36.8	39.9	43.5	OORL	OORL	41.7
LLOQ	25881	33714	33903	34429	37932	35341	35.3	OORL	OORL	29.9	OORL	OORL	OORL
LLOQ	28743	26187	dark	17693	26020	22239	31.1	36.4	INVALID	76.3	OORL	43.9	46.9
LLOQ	28267	31839	35728	21989	28659	26698	31.7	OORL	OORL	55.9	OORL	OORL	OORL
LLOQ	24410	27062	33049	31724	32555	27342	38.0	34.1	OORL	33.5	OORL	OORL	OORL
LLOQ	28661	24940	32018	31023	35478	35904	31.2	39.8	OORL	34.6	OORL	OORL	OORL
LLOQ	25125	26568	23694	28676	28509	29212	36.6	35.4	45.0	38.5	OORL	OORL	38.9
LLOQ	31976	26627	29028	32846	33544	32578	27.4	35.2	30.9	31.9	OORL	OORL	31.4
LLOQ	30451	32170	27318	34697	dark	34732	29.0	OORL	34.7	29.6	INVALID	OORL	INVALID
LLOQ	20367	23691	22855	24851	26913	27527	47.9	43.6	48.0	47.1	OORL	OORL	46.6
LLOQ	16871	18843	21722	23774	dark	22209	61.8	63.2	52.4	50.1	INVALID	44.1	54.3



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LLOQ	24493	30841	30790	28068	32418	29293	37.8	OORL	27.5	39.7	OORL	OORL	OORL
LLOQ	40494	40318	14123	43986	44290	33327	OORL	OORL	106.7	OORL	OORL	OORL	OORL
LLOQ	30872	29914	33496	33811	35181	35405	28.6	27.7	OORL	30.7	OORL	OORL	OORL
LLOQ	21718	32055	33441	34282	27767	27490	44.1	OORL	OORL	30.1	OORL	OORL	OORL
LLOQ	25686	27057	dark	27334	25670	30657	35.7	34.2	INVALID	41.2	OORL	OORL	INVALID
LLOQ	17696	21630	18702	25760	dark	24482	57.8	50.8	67.6	44.7	INVALID	33.3	50.9
LLOQ	33624	33638	37839	33245	26684	30130	OORL	OORL	OORL	32.4	OORL	OORL	OORL
LLOQ	42493	42966	44792	46416	dark	42258	OORL	OORL	OORL	OORL	INVALID	OORL	OORL
LLOQ	27200	33383	33675	32360	35918	32432	33.2	OORL	OORL	32.6	OORL	OORL	OORL
LLOQ	25075	28553	23545	27521	28673	22854	36.7	30.7	45.5	40.8	OORL	40.8	38.9
LLOQ	27649	32001	33694	25077	35458	32971	32.6	OORL	OORL	46.5	OORL	OORL	OORL
LLOQ	27736	33676	32156	26419	33744	27358	32.5	OORL	OORL	43.2	OORL	OORL	OORL
LLOQ	30973	29440	dark	27847	22806	17365	28.5	28.7	INVALID	40.1	48.8	77.0	44.6
LLOQ	27026	29265	29188	30734	33462	30651	33.5	29.1	30.5	35.0	OORL	OORL	32.0
LLOQ	27519	18769	30506	31159	29500	35374	32.8	63.6	28.0	34.3	OORL	OORL	39.7
LLOQ	28777	26831	25267	25583	23803	28188	31.1	34.7	40.1	45.2	39.2	OORL	38.0
LLOQ	22079	25221	28441	30656	33551	25230	43.1	39.0	32.1	35.1	OORL	30.1	35.9
LLOQ	31608	28515	10869	33271	30791	31478	27.8	30.7	163.3	31.4	OORL	OORL	62.8
LLOQ	30592	28711	35830	32018	35982	37523	28.9	30.3	OORL	33.1	OORL	OORL	OORL
LLOQ	20069	26808	28613	31915	dark	35085	48.8	34.8	31.7	33.2	INVALID	OORL	37.1
LLOQ	26415	18544	dark	25260	24409	20325	34.5	64.7	INVALID	46.0	32.9	55.0	46.6
LLOQ	26878	23212	25490	21258	dark	26061	33.7	45.2	39.4	58.6	INVALID	OORL	44.2
LLOQ	25369	29975	32992	25292	dark	15481	36.2	27.6	OORL	45.9	INVALID	95.7	51.3
LLOQ	28697	30922	32079	27393	33552	36578	31.2	OORL	OORL	41.1	OORL	OORL	OORL
LLOQ	24342	30144	29499	31454	30240	27235	38.1	27.3	29.9	33.9	OORL	OORL	32.3
LLOQ	16360	29755	29887	33762	dark	28076	64.5	28.1	29.2	30.7	INVALID	OORL	38.1
LLOQ	25282	19480	24289	27767	29109	30783	36.4	60.0	43.1	40.3	OORL	OORL	44.9
LLOQ	27094	32190	28326	29833	33654	36270	33.4	OORL	32.4	36.5	OORL	OORL	OORL
LLOQ	28707	29171	32732	33932	33864	33271	31.1	29.3	OORL	30.5	OORL	OORL	OORL
LLOQ	24690	25370	dark	26351	25751	27273	37.5	38.6	INVALID	43.4	OORL	OORL	INVALID
LLOQ	28190	31653	31467	35307	36188	29591	31.8	OORL	OORL	28.9	OORL	OORL	OORL
LLOQ	25696	25975	22746	30498	32604	30909	35.6	36.9	48.4	35.4	OORL	OORL	39.1
LLOQ	26504	26704	dark	26832	23333	14721	34.3	35.0	INVALID	42.3	43.8	104.7	38.9
LLOQ	31021	28635	11744	35372	33391	36746	28.4	30.5	142.8	28.8	OORL	OORL	57.6
LLOQ	25137	16839	25970	24435	28695	21368	36.6	74.8	38.1	48.2	OORL	48.7	49.3
LLOQ	27786	32070	32022	32319	29285	30423	32.4	OORL	OORL	32.7	OORL	OORL	OORL

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LLOQ	22812	20346	dark	25324	26246	23558	41.4	56.1	INVALID	45.8	OORL	37.4	45.2
LLOQ	25671	30784	28904	27580	31713	dark	35.7	OORL	31.1	40.7	OORL	INVALID	INVALID

Table 13: 1/2 LLOQ Replicates

SampleID	RLU						Measured concentration (pg/ml)						result
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
1/2 LLOQ	21901	15953	27851	25152	dark	32146	43.6	80.9	33.4	46.3	INVALID	OORL	51.0
1/2 LLOQ	38663	41145	46695	48310	44504	44147	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	33502	26292	27782	32044	30343	33465	OORL	36.1	33.6	33.0	OORL	OORL	OORL
1/2 LLOQ	35275	34778	32116	32665	dark	40598	OORL	OORL	OORL	32.2	INVALID	OORL	OORL
1/2 LLOQ	44083	40341	46801	40502	39023	35949	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	30136	32525	37409	39892	40774	44526	29.4	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	24946	25164	dark	27314	24751	23735	37.0	39.2	INVALID	41.2	29.1	36.6	36.6
1/2 LLOQ	30581	29547	29622	37634	35307	30097	28.9	28.5	29.7	OORL	OORL	OORL	OORL
1/2 LLOQ	38730	35427	39021	37833	39251	42537	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	36920	34778	29899	38555	dark	36078	OORL	OORL	29.1	OORL	INVALID	OORL	OORL
1/2 LLOQ	39039	34582	40885	41988	40707	38342	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	36158	33618	27822	39459	34752	33434	OORL	OORL	33.5	OORL	OORL	OORL	OORL
1/2 LLOQ	28387	20587	34078	32188	dark	36770	31.6	55.0	OORL	32.8	INVALID	OORL	INVALID
1/2 LLOQ	22996	26206	24786	28725	dark	23654	41.8	36.3	41.5	60.8	INVALID	37.0	38.9
1/2 LLOQ	44387	43905	33341	148187	47251	52478	OORL	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	24181	24138	28572	28470	26378	31977	38.4	42.0	31.8	38.9	OORL	OORL	37.8
1/2 LLOQ	32153	36934	39877	41272	41350	41313	27.2	OORL	OORL	OORL	OORL	OORL	OORL
1/2 LLOQ	34531	29162	dark	30400	29961	31694	OORL	29.3	INVALID	35.5	OORL	OORL	OORL
1/2 LLOQ	32158	34330	32760	35565	36369	43286	27.2	OORL	OORL	28.6	OORL	OORL	OORL
1/2 LLOQ	35655	36629	39343	40999	dark	33426	OORL	OORL	OORL	OORL	INVALID	OORL	OORL
1/2 LLOQ	40526	40443	40067	45556	43205	47090	OORL	OORL	OORL	OORL	OORL	OORL	OORL

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Table 14: Analytical Sensitivity Summary


Statistic	Percentage
% of blanks that are OORL	96.7
% of LLOQ that are calculable	55
% of 1/2 LLOQ that are OORL	76.2

10 ANTICOAGULANT COMPARISON

To test the effect of anticoagulants, 20 unique patients donated a total of 3 samples, one EDTA, one Li Heparin, and one Serum. The matched samples were tested in Theranos systems and the results were compared across three matrices. There was no significant difference between serum and EDTA plasma, but the correlation was poor between EDTA plasma and heparin plasma. Both EDTA plasma and serum are suitable for Theranos Estradiol assay. Li-heparin plasma is not recommended to be used as a sample for the Theranos Estradiol assay.

Table 15: EDTA Plasma

EDTA plasma	CLIA conc. (serum) (pg/ml)	RLU						Calculated conc (pg/ml)						result
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
Patient 1	22.6	23601	27147	32211	28534	31433	31505	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 2	49.8	15162	18648	14537	14329	13160	14563	91.3	126.2	125.0	103.3	143.9	109.9	99.6
Patient 3	21	39893	49750	46708	51354	48728	48243	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 4	<20.0	35744	31153	70798	46604	32986	37624	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 5	<20.0	38429	43271	39681	36589	41820	36722	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 6	<20.0	26423	27377	29769	29385	32787	24990	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 7	26.9	12204	14356	14199	15167	17570	16497	140.3	111.4	132.0	93.3	59.0	76.0	84.7
Patient 8	<20.0	31243	28315	26432	25099	27274	22302	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 9	34.3	20088	22438	20177	23338	23479	26964	40.9	OORL	35.0	33.3	OORL	OORL	OORL
Patient 10	144	4531	9713	6680	12906	11189	10783	737	258	464	124	212	211	186
Patient 11	24	16440	8362	37591	37530	36068	36312	75.5		OORL	OORL	OORL	OORL	OORL
Patient 12	20.4	15117	17418	18210	18576	20943	20961	91.9	65.1	62.4	62.2	OORL	OORL	52.3
Patient 13	27	15379	13717	15218	15110	16377	11535	88.4	124.7	111.6	93.9	75.9	185.8	81.5
Patient 14	74.2	14484	15050	15085	14142	12817	11284	100.7	98.6	114.2	105.7	153.8	193.9	87.5
Patient 15	50.9	10622	10687	9833	10873	11227	12164	178.6	215.4	261.4	164.2	210.1	166.9	184.5
Patient 16	35.2	19316	18478	13449	19104	20068	21008	47.3	54.1	148.6	58.4	32.7	OORL	29.5
Patient 17	<20.0	25004	33095	34293	38189	39896	39819	OORL	OORL	OORL	OORL	OORL	OORL	OORL

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Patient 18	111	9214	2288	10014	9880	9766	8048	226	1220	254	192	282	348	224
Patient 19	24.9	21720	27226	27100	29489	27921	23907	28.5	OORL	OORL	OORL	OORL	OORL	OORL
Patient 20	24	20405	22539	24333	22116	26355	25722	38.4	OORL	OORL	39.7	OORL	OORL	OORL

Table 16: Li Heparin Plasma

Li-Hep	CLIA conc. (serum) (pg/ml)	RLU						Calculated conc (pg/ml)						result
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
Patient 1	22.6	22006	6589	25164	23742	24308	21685	OORL	OORL	31.3	OORL	OORL	OORL	OORL
Patient 2	49.8	13416	15495	13674	14389	13005	15095	11.8	91	143	103	148	100	100
Patient 3	21	32418	39501	28006	31848	31863	15522	OORL	OORL	OORL	OORL	OORL	92.0421	OORL
Patient 4	< 20.0	34172	54992	54337	55858	49609	31335	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 5	< 20.0	28223	24566	26110	28466	25135	25564	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 6	< 20.0	31826	34269	32267	37630	42296	40198	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 7	26.9	15699	17921	20271	20171	19585	18772	84.4	59.7	83.7	51.2	37.3	44.6	33.3
Patient 8	< 20.0	29446	31747	26090	33809	34637	26802	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 9	34.3	6970	7231	36179	34441	32164	21706	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 10	144	12400	10082	10954	11437	10187	8950	136	>241	219	151	259	293	202
Patient 11	24	22445	21828	25023	22906	23115	22208	OORL	30.0	OORL	35.5	OORL	OORL	OORL
Patient 12	20.4	20255	19808	20636	21134	22513	20017	39.5	42.9	28.8	45.3	OORL	30.1	18.4
Patient 13	27	17041	18172	21577	16611	18126	14948	68.9	57.1	OORL	78.5	52.2	102.5	53.8
Patient 14	74.2	11970	12577	11428	13196	12831	10830	145	153	203	119	153	210	148
Patient 15	50.9	13553	12449	11884	11709	9197	12884	115	156	189	145	317	148	135
Patient 16	35.2	11970	12577	11428	13196	12831	10830	145	153	203	119	153	210	148
Patient 17	< 20.0	24705	23302	24852	23997	32912	20198	OORL	OORL	OORL	30.1	OORL	28.1	OORL
Patient 18	111	9911	10265	11115	2487	12346	12471	200	233	213	OORH	169	158	180
Patient 19	24.9	21096	24924	23337	25938	28356	24734	33.1	OORL	OORL	OORL	OORL	OORL	OORL
Patient 20	24	17119	15192	16465	18893	22471	21189	68.1	96.1	89.5	59.9	OORL	OORL	60.5

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Table 17: Serum

Serum SampleID	CLIA conc. (serum) (pg/ml)	RLU						Calculated conc (pg/ml)						result
		Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	
Patient 1	22.6	27146	35490	26897	38647	38632	37237	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 2	49.8	14284	13628	13957	15021	15596	14458	103.7	126.7	137.2	94.9	89.0	112.0	93.5
Patient 3	21	35966	43020	41641	45259	52613	59189	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 4	< 20.0	77362	77027	79238	80653	70494	69550	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 5	< 20.0	26898	41341	29462	33186	29775	32044	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 6	< 20.0	31727	34949	36655	36359	40536	42601	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 7	26.9	15983	16893	16434	18761	17122	19862	80.9	71.4	90.0	60.9	65.0	31.8	48.5
Patient 8	< 20.0	24853	28096	29287	30362	24160	28216	OORL	OORL	OORL	OORL	OORL	51.56281	OORL
Patient 9	34.3	18822	6599	21988	24836	25951	19487	51.6		OORL	OORL	OORL	36.1	OORL
Patient 10	144	12005	12074	10624	11294	11655	13102	145	167	230	154	193	142	156
Patient 11	24	29264	5407	33774	32705	31213	36730	OORL		OORL	OORL	OORL	OORL	OORL
Patient 12	20.4	13819	19789	21692	18485	16268	19470	110.9	43.0	OORL	62.9	77.6	36.3	47.9
Patient 13	27	17720	21144	20928	23332	9440	18846	62.0	33.9	OORL	33.3	301.7	43.6	24.5
Patient 14	74.2	14601	13177	15474	17396	16680	11020	99.0	137.2	106.9	71.6	71.3	202.9	97.8
Patient 15	50.9	6143	11079	16853	24853	11298	12867	430.7	200.5	83.2	OORL	207.2	147.9	199.3
Patient 16	35.2	18488	10721	17398	18546	18772	18887	54.6	214.1	74.6	62.5	45.0	43.1	37.5
Patient 17	< 20.0	24216	22486	25260	27023	25131	23485	OORL	OORL	OORL	OORL	OORL	OORL	OORL
Patient 18	111	7287	8651	10334	10612	11354	12446	327	316	241	171	205	159	223
Patient 19	24.9	28263	6127	28836	32160	32004	33852	OORL		OORL	OORL	OORL	OORL	OORL
Patient 20	24	18501	19486	20401	20933	17898	24016	54.5	45.4	32.0	46.5	54.9	OORL	28.0

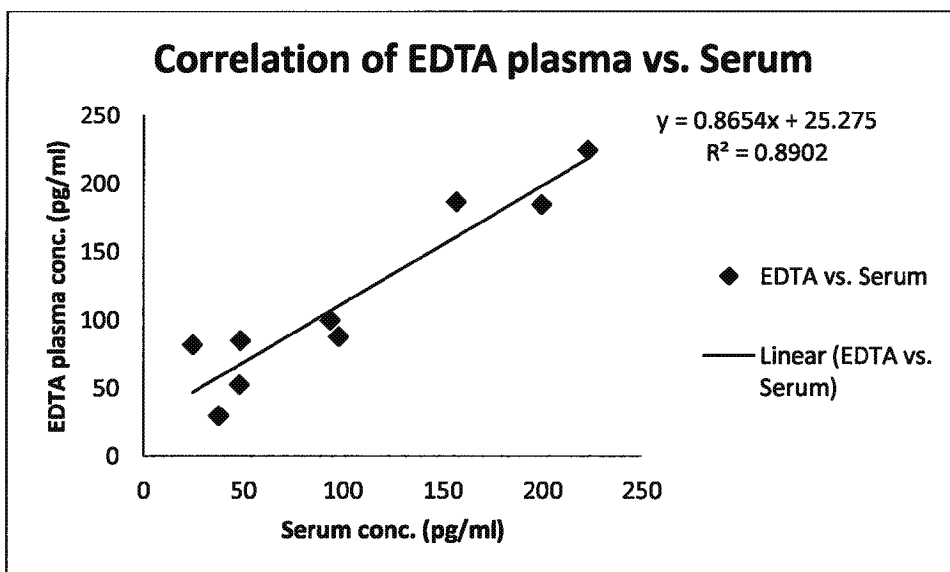
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Table 18: Summary of EDTA v. Li Heparin v. Serum

SampleID	CLIA conc. (serum) (pg/ml)	Theranos results (pg/ml)		
		serum conc.	EDTA plasma conc.	Heparin plasma conc.
Patient 1	22.6	OORL	OORL	OORL
Patient 2	49.8	93.5	99.6	100
Patient 3	21	OORL	OORL	OORL
Patient 4	< 20.0	OORL	OORL	OORL
Patient 5	< 20.0	OORL	OORL	OORL
Patient 6	< 20.0	OORL	OORL	OORL
Patient 7	26.9	48.5	84.7	33.3
Patient 8	< 20.0	OORL	OORL	OORL
Patient 9	34.3	OORL	OORL	OORL
Patient 10	144	156	186	202
Patient 11	24	OORL	OORL	OORL
Patient 12	20.4	47.9	52.3	18.4
Patient 13	27	24.5	81.5	53.8
Patient 14	74.2	97.8	87.5	148
Patient 15	50.9	199.3	184.5	135
Patient 16	25.2	37.5	29.5	148
Patient 17	< 20.0	OORL	OORL	OORL
Patient 18	111	223	224	180
Patient 19	24.9	OORL	OORL	OORL
Patient 20	24	28.0	OORL	60.5

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Figure 8: EDTA Plasma v Serum



11 INTERFERENCE

Matrix interference was evaluated by preparing hemolyzed, icteric and lipemic matrix and spiking three concentration levels of analyte into prepared matrix respectively. The spiked serum samples were measure on the Theranos System and predicate. The recovery was calculated with measured values from Theranos system and from predicate method.

Icteric samples with Bilirubin at 5mg/dL and lipemic samples with Triglycerides at 250mg/dL didn't show significant difference on Estradiol results. Hemolyzed samples with 250mg/dL Hemoglobin had slightly low recovery so results of hemolyzed samples in Theranos system should be interpreted with caution for Estradiol assay.

Table 19: Results of Hemolyzed Samples – 250 mg/dL Hemoglobin

Spiked sample (pg/ml)	RLU						Calculated conc. (pg/ml)						% recovery	
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6		result
695	5240	5614	4513	6112	6694	6549	472.1	476.5	1070.3	413.6	431.2	399.3	429.5	61.8
267	10484	dark	11629	10983	10023	11833	169.5		157.0	187.4	226.7	166.1	166.0	62.2

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	14842	14260	15115	18689	19514	19016	105.6	114.3	99.2	99.0	90.0	86.2	81.7	65.3
20.3	43969	42009	49651	40371	54293	52488	OORL	OORL	OORL	OORL	OORL	OORL	OORL	--

Table 20: Icteric Samples – 5 mg/dL Bilirubin

Spiked sample (pg/ml)	RLU						Calculated conc. (pg/ml)							% recovery
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	result	
639	4465	5211	4559	5364	4870	6121	904.1	714.7	764.3	680.4	773.0	437.2	731.3	114.4
253	4589	8140	8759	dark	9444	9675	854.4	341.3	324.1		261.2	243.9	280.0	110.7
117	12168	13530	15549	16326	12626	16989	175.0	174.1	144.5	132.6	176.1	120.7	137.8	117.8
<20	36581	42861	42491	48414	46551	48001	31.8	OORL	OORL	OORL	OORL	OORL	OORL	--

Table 21: Lipemic Samples – 250 mg/dL Triglycerides

Spiked sample (pg/ml)	RLU						Calculated conc. (pg/ml)							% recovery
	Tip1	Tip2	Tip3	Tip4	Tip5	Tip6	conc1	conc2	conc3	conc4	conc5	conc6	result	
535	4835	4915	5082	5107	4607	5358	426.5	497.8	488.3	511.2	664.9	557.0	517.3	96.7
189	8090	6908	8383	8096	8941	8504	199.9	291.0	243.9	254.7	230.1	267.8	234.2	123.9
79.1	12813	16555	15664	18348	14585	18647	108.9	82.5	111.4	90.8	119.1	84.6	82.2	103.9
<20	33069	36837	40644	35615	39143	43260	OORL	OORL	OORL	OORL	OORL	OORL	OORL	--

12 CROSSREACTIVITY

Crossreactivity test data were obtained from assay development report. The cross-reactants were prepared in buffer to avoid other interference in serum or plasma. The recovery was calculated to determine the effect of the substance. No significant cross reactivity showed in tested substances and the results are comparable to predicate method.

Table 22: Cross-reactive test

Test Substance	Tested Conc pg/mL	Recovered Conc pg/mL	% Cross Reactivity
Estrone	5000	156	3.12
Estriol	40,000	20	0.05
Progesterone	70,000	OORL	0.00
Cortisol (Hydrocortisone)	2000	OORL	0.00

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13 ANALYTE STABILITY

The stability of analyte was evaluated at 4C and room temperature. Aliquots of calibrators made by spiking Estradiol in depleted serum were stored at -80C. Multiple sets of stability testing calibrators were then transferred to either room temperature or 4C at the 0hr time point and stored at these temperatures for the remainder of testing. At each time point, one set of calibrator was analyzed and back calculated from calibration curve obtained at 0hr time point.

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

Table 23: Analyte Stability Summary: 4C

Level (pg/ml)	RLU						Calculated Concentration (pg/ml)					
	0hr	1hr	4hr	12hr	24hr	72hr	0hr	1hr	4hr	12hr	24hr	72hr
1293.5	3877	3590	3290	3543	3714	3498	OORH	OORH	OORH	OORH	OORH	OORH
323.4	7221	7803	5788	6908	7027	8876	294.1	253.7	399.2	262.8	279.9	209.0
53.9	24852	24586	23861	16986	24326	21341	26.4	62.2	28.1	43.3	26.0	26.7

Table 24: Analyte Stability Summary: RT

Level (pg/ml)	RLU						Calculated Concentration (pg/ml)					
	0hr	1hr	4hr	12hr	24hr	72hr	0hr	1hr	4hr	12hr	24hr	72hr
1293.5	3877	4273	3936	4481	3491	3604	OORH	823.3	OORH	835.3	OORH	OORH
323.4	7221	6588	5761	7995	7799	7171	294.1	317.3	475.0	259.4	236.9	293.8
53.9	24852	24303	19621	25693	28964	26006	26.4	29.8	50.9	56.2	OORL	23.3


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Figure 9: Analyte Stability at 4C

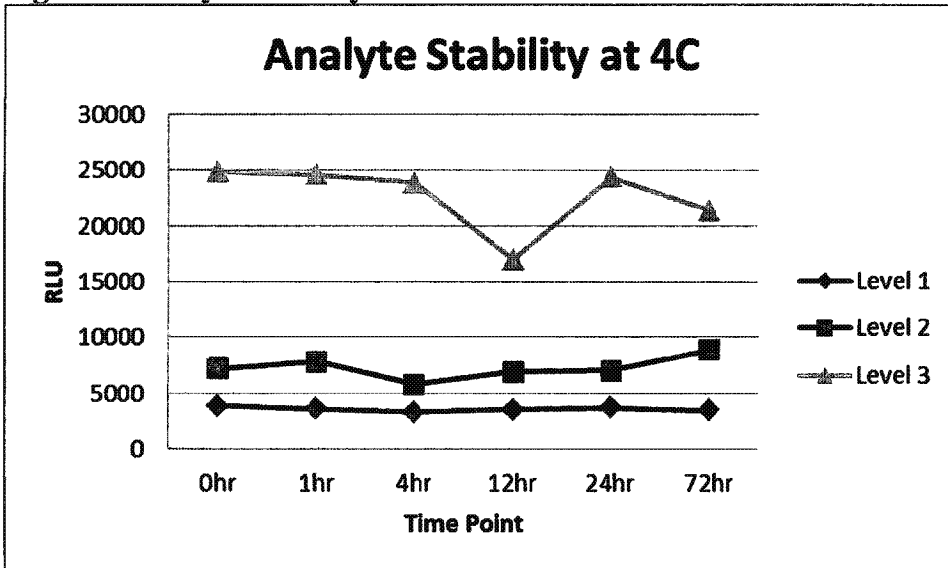
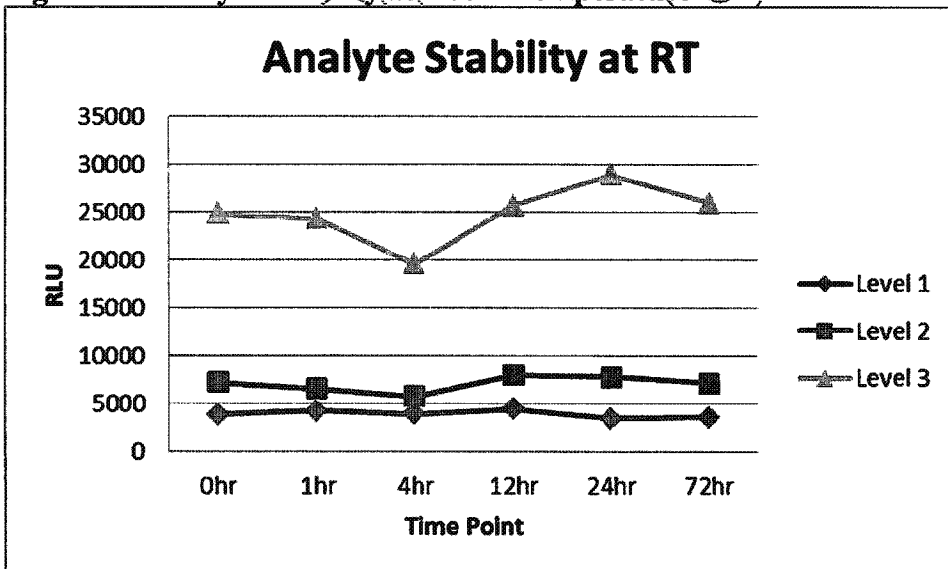


Figure 10: Analyte Stability at Room Temperature



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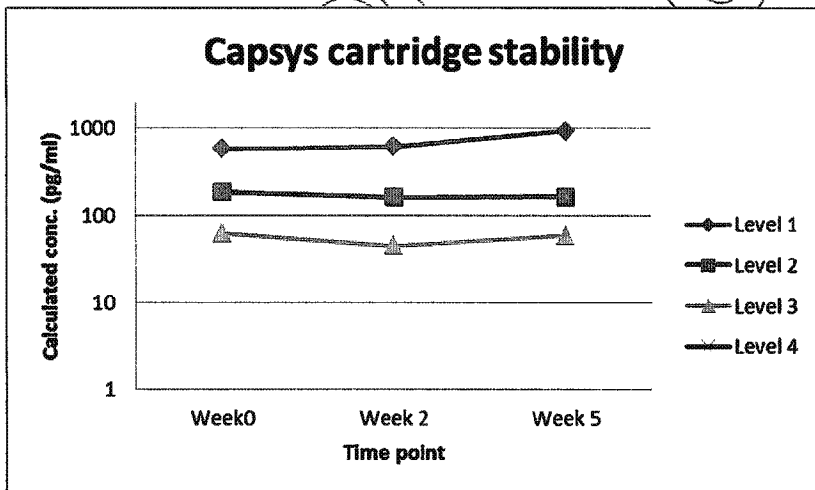
14 Reagent Stability in Capsys Cartridges

Reagent stability was conducted with Capsys built cartridges. Cartridges with all reagents filled by Capsys were stored at 4C and the monitoring of assay performance is on-going. At each time point, a set of calibrators made by spiking Estradiol in depleted serum was analyzed using Capsys cartridges and back calculated from calibration curve obtained at 0 time point. Current data shows reagents in Capsys cartridges are stable after 5 weeks' storage.

Table 25: Capsys cartridge stability

	Nominal conc (pg/ml)	Calculated conc (pg/ml)		
		Week0	Week 2	Week 5
Level 1	646.7	585	622	939
Level 2	161.7	187	162	165
Level 3	53.9	62.0	44.3	58.3
Level 4	0	OORL	OORL	OORL

Figure 11: Capture antibody working solution stability at 4C



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