From: Kapil Gadkar <kgadkar@theranos.com>
Sent: Monday, July 26, 2010 7:27 PM
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Subject: Validation reports for 3 PD markers: LH, FSH & Estradiol
Attach: Theranos_FSH Validation report.pdf; LH Assay validation report.pdf; Theranos_Estradiol_Validation report.pdf

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Hello all,

Attached plz find the Assay Validation Reports for the 3 PD markers of LH, FSH & Estradiol. Plz note the confidentiality information on the first page of each of these reports.

Plz let me know if you have any questions regarding the reports.

Kind regards.

Kapil Gadkar
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Follicle-Stimulating Hormone Assay Validation Report

Theranos, Inc.

July 22, 2010

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1. Analyte Background

Follicle-stimulating hormone (FSH) is a hormone synthesized and secreted by gonadotropes in the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the human body. Analyte concentration reference ranges from the literature:

FEMALES:	mIU/mL
Follicular phase	3.5 - 12.5
Ovulation phase	4.7 - 21.5
Luteal phase	1.7 - 7.7
Postmenopause	25.8 - 134.8
MALES:	1.5 - 12.4

2. Assay Specifications

The Theranos assay for FSH is a sandwich ELISA usable with human whole blood, plasma and serum. The reportable range for the assay is 200-3 mIU/mL.

3. Reference Assays and Standards

The following commercial ELISA kit has been used in house as a reference assay:

Genway FSH-Elisa Kit Catalog# 40-052-115017
Reported range: 200-5 mIU/mL

Standardization: Theranos FSH standards are calibrated against the World Health Organization's First International Standard for Follicle Stimulating Hormone, Recombinant Human FSH (Code 92/642).

4. Cross Reactivity

The FSH assay was tested for cross reactivity with Luteinizing Hormone (LH) and human Chorionic Gonadotropin (hCG). Cross reactivity was determined by testing the analytes mentioned below with the finalized antibody pair.

Result: No significant cross reactivity was observed.

Test Substance	Test Substance Level	% Cross Reactivity
LH	20 ng/mL	0.83
hCG	216 ng/mL	0.06

5. Interfering Substances

The FSH assay was tested for interference to Luteinizing Hormone and human Chorionic Gonadotropin . The test substances were added to FSH calibrators in assay buffer at levels higher than the expected clinical concentrations of these test substances, and the impact on recovery of FSH was measured.
Result: No significant interference was observed, recovery of FSH was within 10 % of nominal levels in the mid range of the assay.

Test Substance	Test Substance Level	Nominal [FSH] mIU/mL	Recovered [FSH] mIU/mL	% Recovery
LH	60 ng/mL	40.0	44.0	110
hCG	648 ng/mL	40.0	43.5	109

6. Precision

Inter-Reader Precision

Inter-reader Precision test was evaluated by running a single analyte level (2 mIU/mL) on 24 instruments.
Result: Total Inter-Reader concentration CV % at 2 mIU/mL was 14%

Cartridge #	Recovered Conc. mIU/mL
1	2.1
2	1.6
3	2.1
4	2.4
5	2.1
6	2.3
7	1.6
8	1.9
9	2.0
10	2.6
11	2.0
12	2.1

Cartridge #	Recovered Conc. mIU/mL
13	2.1
14	2.3
15	1.9
16	2.6
17	2.0
18	1.8
19	2.1
20	2.0
21	1.9
22	2.1
23	2.6
24	2.7

	Recovered [FSH] mIU/mL			
Nominal [FSH] mIU/mL	Mean Conc.	StDev	CV %	% Recovery
2.0	2.1	0.30	14	106

Inter-Cartridge Lot Precision

Precision of the assay across multiple reagent lots was evaluated by running a 6 point standard curve on three different reagent lots over multiple instruments, 3 cartridges per point.

The Average inter-lot concentration CV was 7.7 %.

FSH: Concentration CV %

		Conc mIU/mL			Total Concentration CVs			
[FSH]	Cartridge	Lot 1	Lot 2	Lot 3	Mean	StDev	CV %	Average% Recovery
200	1	197.6	200.9	200.2	199.5	1.3	0.6	100
	2	200.7	198.8	199.4				
	3	201.2	198.2	198.6				
40	1	44.0	43.1	37.4	40.3	3.9	9.7	101
	2	42.2	43.6	32.1				
	3	42.2	40.7	37.5				
8	1	8.4	7.6	6.9	7.9	0.9	11.4	98
	2	9.3	7.2	7.0				
	3	8.9	7.1	8.4				
2	1	2.1	1.9	2.2	2.0	0.2	7.8	102
	2	1.7	2.0	2.0				
	3	2.1	2.0	2.3				
0.5	1	0.5	0.5	0.5	0.5	0.0	5.0	100
	2	0.5	0.5	0.5				
	3	0.5	0.5	0.5				
0	1	0.3	0.3	0.3	0.3	0.0	3.5	-
	2	0.3	0.3	0.3				
	3	0.3	0.3	0.3				

7. Control Comparison

a) On the Genway ELISA kit

The commercially available analyte used for the Theranos assay development was tested on the Genway Elisa Kit Catalog # 40-052-1150171.

Result: Recovery was within an acceptable range.

The commercially available analyte used by the Theranos assay was tested on the Genway FSH ELISA kit

Nominal [FSH] mIU/mL	Recovered [FSH] mIU/mL			
	Mean Conc	StDev	CV%	% Recovery
196	221.3	12	5	113
98	92.6	10	11	94
32	38.3	0	1	120
16	18.3	0	1	114
8.0	8.4	1	9	107
4	4.1	0	3	104
2	OORL	-	-	-
1	OORL	-	-	-
0.5	OORL	-	-	-
0	OORL	-	-	-

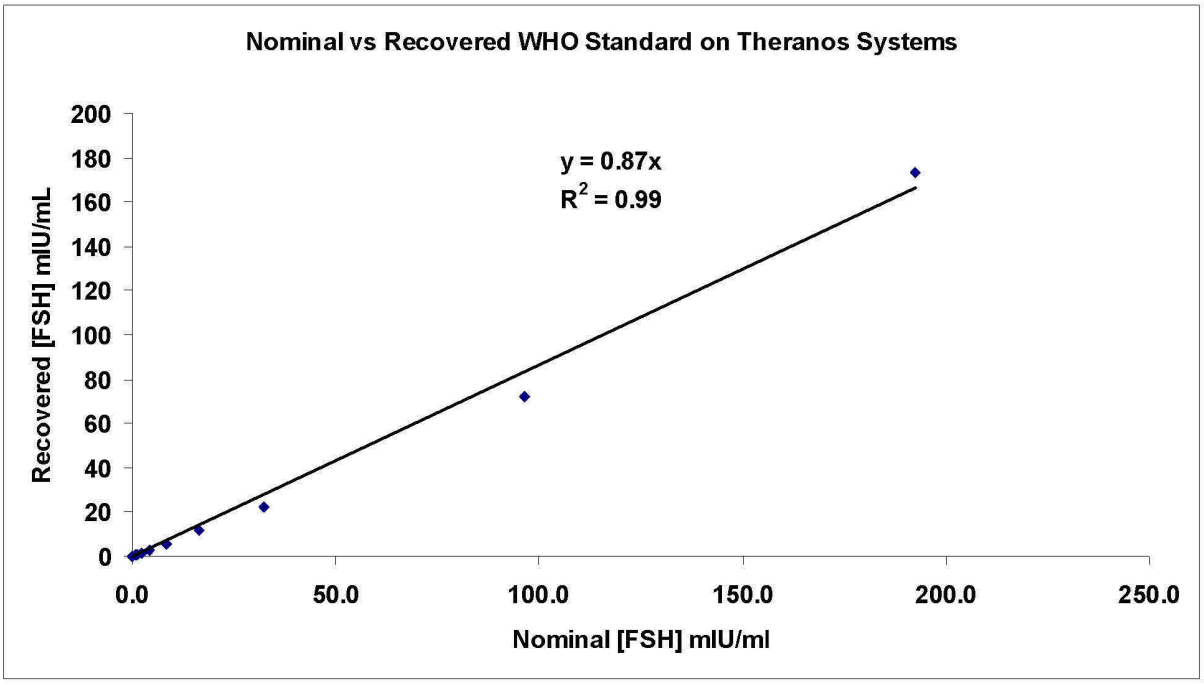
OORL: Out of Range Low

b) WHO Standard Recovery

Recovery of the NISBC WHO FSH standard (NIBSC 92/642) on the Theranos System was tested.
Result: Average % recovery was 87 % of nominal. Therefore the Theranos System was subsequently recalibrated to the WHO standard.

WHO Standard curve in Assay Buffer

[FSH]	Signal (RLU)			Recovered [FSH] mIU/mL			
mIU/mL	Mean RLU	StDev	% CV	Mean Conc.	StDev	CV%	% Recovery
192	139340	9045	6	174	9	5	90
96	52331	2796	5	72	4	5	75
32	18371	425	2	23	1	3	70
16	10974	960	9	12	1	11	75
8	5931	74	1	6	0.1	2	69
4	3773	228	6	3	0.2	8	78
2	1737	124	7	1.2	0.1	9	60
1	1131	84	7	0.7	0.1	9	72
0.5	788	59	7	0.5	0.0	8	96
0	451	58	13	0.3	0.0	13	

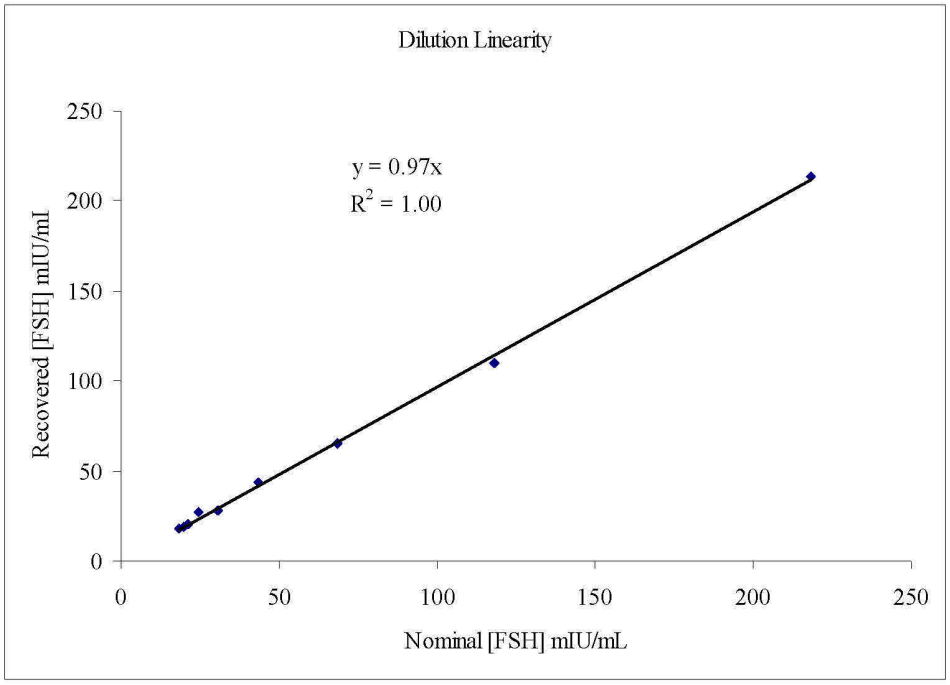


8. Dilution Linearity

A sample with a low endogenous serum FSH level was spiked with 200 mIU/mL FSH and recombined with the low FSH starting sample to determine dilution linearity. Calibration was performed using an assay buffer standard curve.

Result: The % recovery ranged from 85-110%.

	Signal (RLU)			Recovered [FSH] mIU/mL			
Nominal [FSH] mIU/mL	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
218	292013	29468	10	240	35	15	110
118	159516	8802	6	104	7	7	88
68	97912	2200	2	59	1	2	87
43	67091	7264	11	39	4	11	92
30	43113	1625	4	26	1	4	85
24	41943	2208	5	25	1	5	104
21	32116	1124	4	20	1	3	94
19	30275	1283	4	19	1	4	96
17.7	28793	846	3	18	0	3	100



9. Whole Blood Spike Recovery

Whole blood samples were spiked with FSH at different levels across the assay range then analyzed on the Theranos System, calibrated on assay buffer calibrators. The nominal values for FSH were computed by measuring the endogenous level and adding it to the spike level. Recovery was excellent for all samples.

Whole Blood Sample 1

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	193.3	121365	8199	7	175.7	22	13	91
96	97.3	85217	4275	5	96.1	8	8	99
32	33.3	32078	4375	14	26.9	4	15	81
16	17.3	17334	774	4	14.4	1	4	84
8	9.3	9121	899	10	7.9	1	9	85
0	1.3	1891	138	7	1.3	0	11	

Whole Blood Sample 2

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	193.0	128529	7126	6	195.2	20	10	102
96	97.0	87216	9623	11	100.3	18	18	104
32	33.0	33875	1900	6	28.5	2	6	89
16	17.0	17324	812	5	14.4	1	4	90
8	9.0	9464	622	7	8.2	1	6	102
0	1.0	1680	67	4	1.0	0	6	

Whole Blood Sample 3

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	192.5	124989	10090	8	185.8	27	15	97
96	96.5	93644	5614	6	111.9	11	10	117
32	32.5	37191	3648	10	31.7	3	11	99
16	16.5	19091	466	2	15.8	0.3	2	99
8	8.5	8619	465	5	7.5	0.4	5	94
0	0.5	1151	84	7	0.5	0.0	14	

10. Matrix Effects

The impact of various sample matrixes was evaluated on the assay by spiking in FSH across the range of the assay into commercially obtained hemolyzed, lipemic and icteric sera. Recovery of spiked samples was calculated on an assay buffer standard curve.

Result: Recovery was within the acceptable range for all tested matrixes.

Spiked into Hemolyzed Serum

[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	163.9	163.6	2	1	100
80.0	83.9	86.7	6	7	103
40.0	43.9	44.8	3	6	102
20.0	23.9	25.8	1	5	108
10.0	13.9	12.1	0	4	87
0.0	3.9	3.9	0	9	100

Spiked into Lipemic Serum

[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	164.5	160.9	6	4	98
80.0	84.5	95.1	11	11	113
40.0	44.5	47.5	6	12	107
20.0	24.5	25.5	2	9	104
10.0	14.5	17.9	2	13	124
0.0	4.5	4.5	1	15	-

Spiked into Icteric Serum

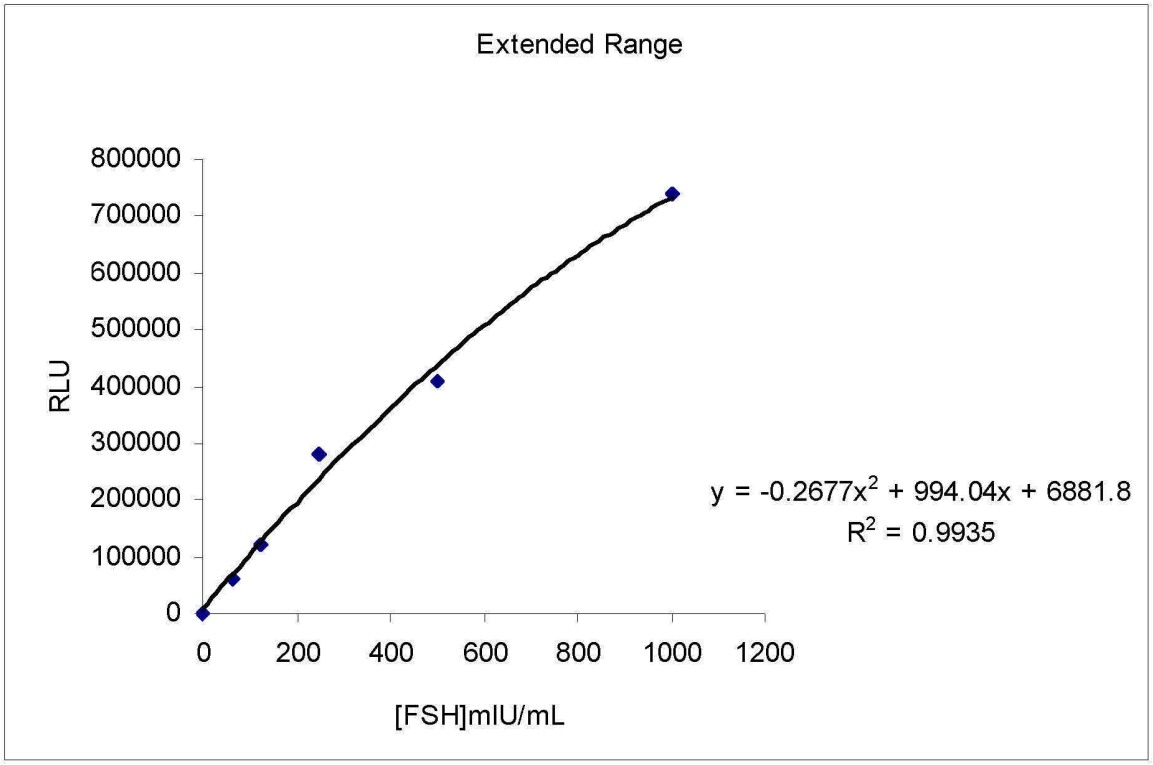
[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	163.5	154.3	7	5	94
80.0	83.5	83.2	4	5	100
40.0	43.5	53.4	2	5	123
20.0	23.5	20.0	2	8	85
10.0	13.5	16.5	1	9	122
0.0	3.5	3.5	0	14	-

11. Extended Range

A standard curve with levels up to five times the normal range was run to check for a high dose hook effect.

Result: No hook effect was observed.

[FSH]mIU/mL	Mean RLU	StDev	CV %
1000	737502	60311	8
500	410291	8450	2
250	278857	4201	2
125	123189	3070	2
63	60790	3771	6
0	547	27	5



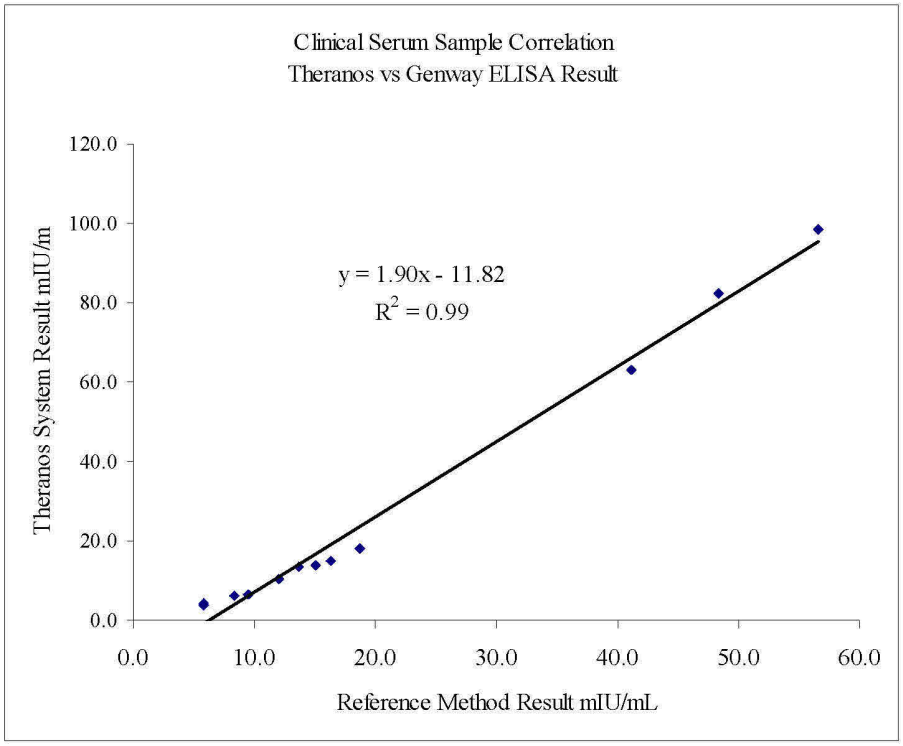
12. Validation in Clinical Samples

FSH assay was validated by testing 18 clinical samples from different stages of the menstrual cycle, pregnancy and postmenopausal patients on the Theranos System and on the Genway FSH ELISA (Cat #40-0521150171).

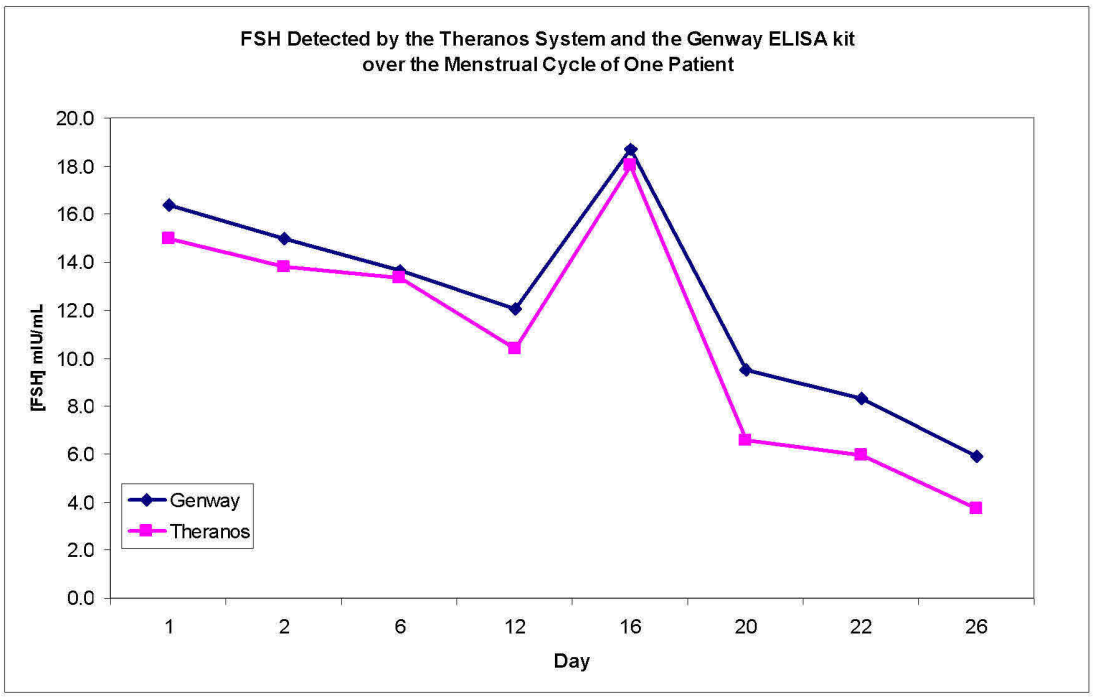
Result: Correlation of the Theranos result to Genway result was R^2=0.99.

Sample ID	Sample Type	Genway Mean [FSH] mIU/mL	Theranos System [FSH] mIU/mL		
			Mean Conc.	Std.Dev.	CV %
1	Menstrual	16.4	15.0	2.5	16
2	Menstrual	15.0	13.8	1.2	9
6	Menstrual	13.6	13.3	1.6	12
12	Menstrual	12.0	10.4	1.5	14
16	Menstrual	18.7	18.1	2.4	13
20	Menstrual	9.5	6.6	1.1	16
22	Menstrual	8.3	6.0	0.6	11
26	Menstrual	5.9	3.7	0.2	5
50	Pregnancy	OORL	OORL	-	-
51	Pregnancy	OORL	OORL	-	-
53	Pregnancy	1.6	OORL	-	-
54	Pregnancy	OORL	OORL	-	-
56	Pregnancy	5.8	4.2	0.8	20
62	Pregnancy	6.9	OORL	-	-
63	Pregnancy	10.6	OORL	-	-
80	Post-Menopausal	41.1	63.1	6.5	10
81	Post-Menopausal	56.6	98.4	6.6	7
82	Post-Menopausal	48.3	82.4	6.5	8

OORL: Out of Range Low



For a congruent set of samples from a menstrual cycle, we compared results for the Genway and Theranos systems. There was excellent agreement of both the absolute values and of the trends in analyte concentration over time.



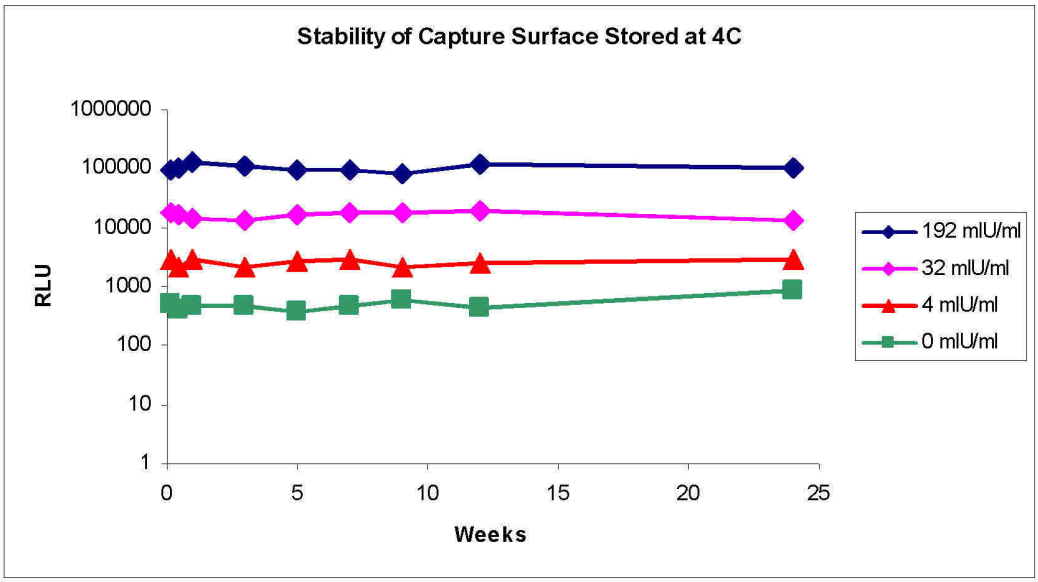
13. Stability

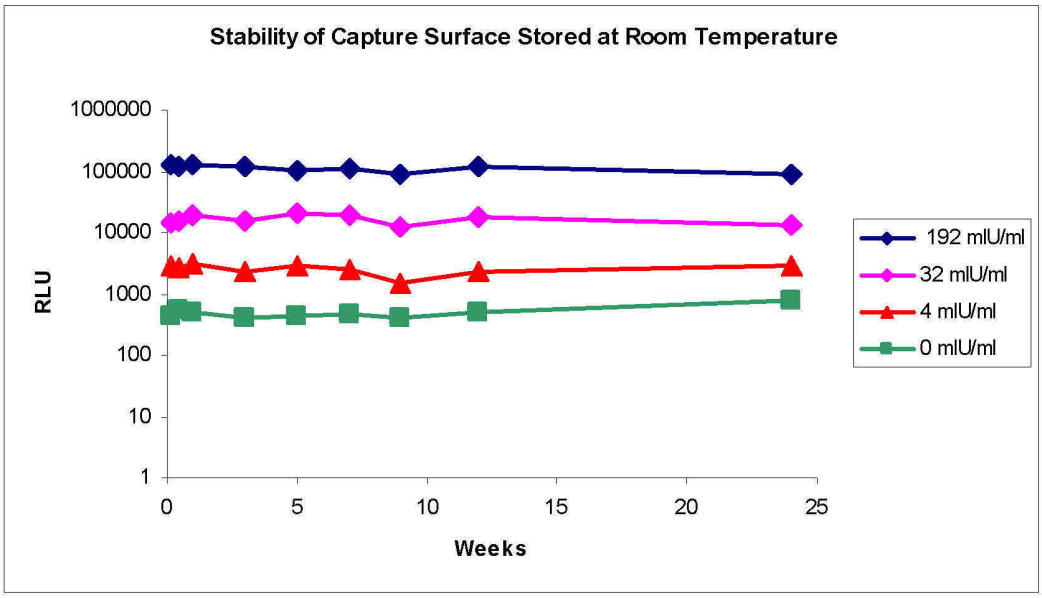
For each stability time point, all reagents are formulated fresh except the test reagent. In addition, a control is included with all reagents formulated fresh from stock materials.

Result: The capture surface and detection antibody show good stability up to 24 weeks.

Capture Surface Stability

Time point	FSH mIU/mL	Control		Stored at 4°C		Stored at Room Temp.	
		Mean RLU	CV %	Mean RLU	CV %	Mean RLU	CV %
Day 1	192	89334	8	92443	13	131813	5
	32	15078	7	17729	6	14228	11
	4	1626	10	2821	4	2944	3
	0	248	9	494	7	431	7
Week 1	192	119356	10	125498	7	124741	8
	32	13787	14	14762	16	19686	18
	4	2358	9	2827	3	3060	10
	0	352	1	479	6	495	10
Week 3	192	93480	5	109203	19	116024	11
	32	13596	11	13037	11	15419	33
	4	1862	10	2158	11	2371	15
	0	277	5	456	9	403	10
Week 5	192	100002	11	95136	11	101719	11
	32	17714	15	16252	8	21310	19
	4	3067	6	2615	14	2978	5
	0	456	9	379	7	417	9
Week 7	192	97913	12	94121	12	108133	20
	32	18719	7	17873	19	19233	26
	4	2642	22	2991	10	2468	19
	0	468	19	468	7	471	5
Week 9	192	75819	20	84401	1	90826	4
	32	12586	11	17242	8	12079	6
	4	2476	17	2212	21	1547	5
	0	549	3	578	10	414	5
Week 12	192	124197	10	116690	9	118224	9
	32	20272	22	19697	16	18190	24
	4	2998	6	2542	11	2333	10
	0	501	8	446	7	483	6
Week 24	192	90320	8	101466	5	91124	10
	32	15935	10	13443	8	13553	21
	4	2541	9	2937	9	2834	6
	0	1096	2	889	11	750	6

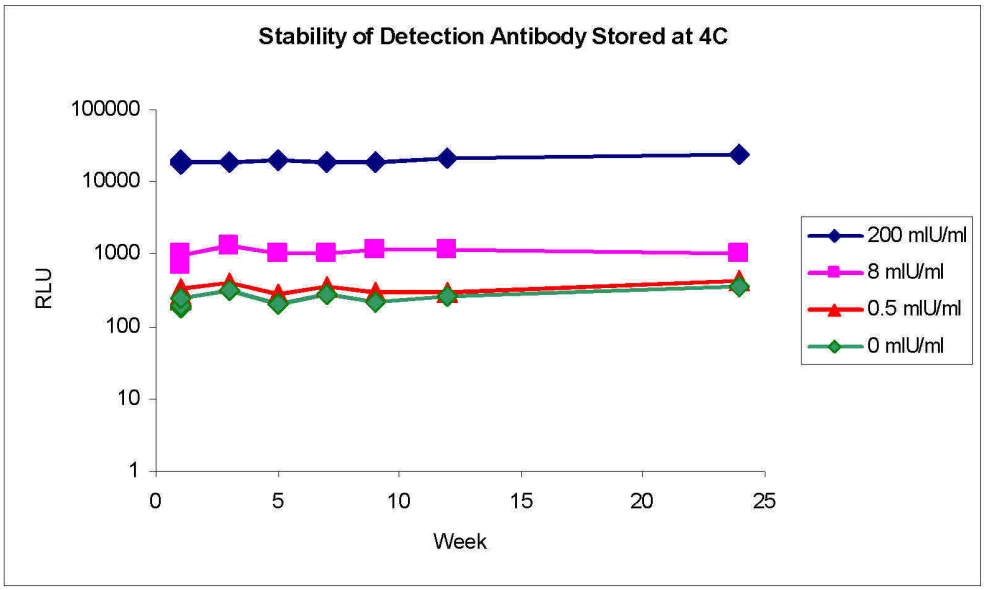

Stability of Capture Surface Stored at 4C
RLU
1000000
100000
10000
1000
100
10
1
0
5
10
15
20
25
Weeks
192 mIU/ml
32 mIU/ml
4 mIU/ml
0 mIU/ml

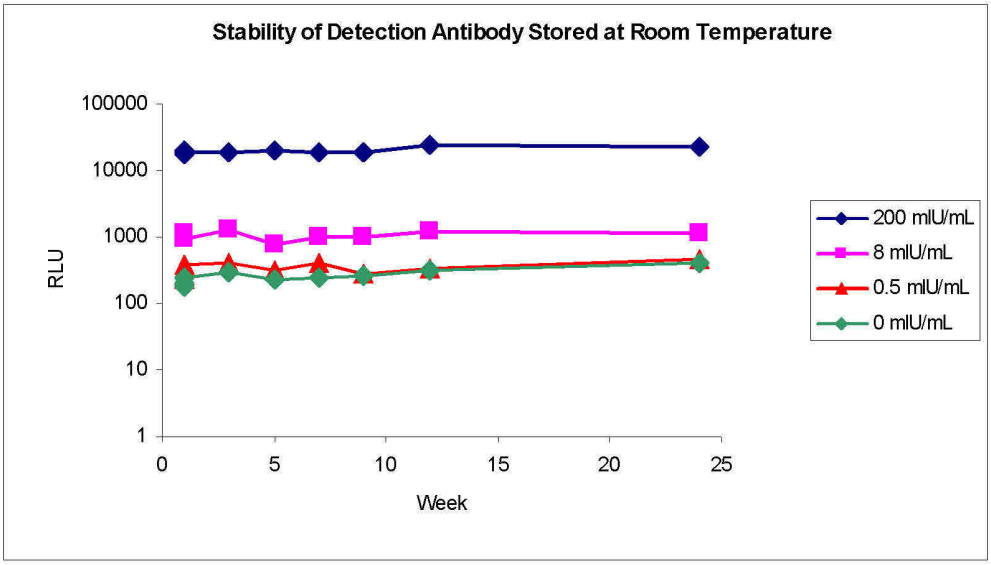

Stability of Capture Surface Stored at Room Temperature
RLU
1000000
100000
10000
1000
100
10
1
0
5
10
15
20
25
Weeks
192 mIU/ml
32 mIU/ml
4 mIU/ml
0 mIU/ml

Detection antibody stability was monitored on a microtitre plate.

Detection Antibody Stability

Time point	[FSH] mIU/mL	Control		Stored at 4°C		Stored at Room Temp.	
		Mean RLU	CV %	Mean RLU	CV %	Mean RLU	CV %
Day 1	200	19675	1	20323	4	20019	4
	8	977	10	826	4	934	8
	0.5	242	9	228	6	329	25
	0	225	11	183	11	208	7
Week 1	200	17256	2	18154	4	17626	5
	8	932	7	718	8	1156	13
	0.5	241	6	229	10	226	14
	0	147	9	193	1	178	4
Week 3	200	18578	2	18674	1	18406	3
	8	831	2	947	4	963	10
	0.5	355	14	336	3	377	5
	0	276	8	248	16	251	14
Week 5	200	18605	1	19202	8	19324	4
	8	914	3	1343	4	1301	7
	0.5	383	2	398	9	399	4
	0	349	2	318	5	300	15
Week 7	200	20689	11	20606	7	20724	2
	8	936	14	1007	4	759	13
	0.5	328	10	273	30	324	11
	0	187	7	211	12	228	21
Week 9	200	19460	4	19055	3	18829	1
	8	1000	7	1014	7	1029	7
	0.5	345	30	369	15	400	10
	0	247	13	276	11	251	24
Week 12	200	18981	5	18766	3	18794	3
	8	1215	10	1157	14	983	8
	0.5	377	14	304	14	277	11
	0	274	23	220	12	260	8
Week 24	200	23775	2	21881	4	23716	1
	8	1242	7	1178	6	1212	7
	0.5	331	16	306	12	343	3
	0	250	11	264	6	321	11


Stability of Detection Antibody Stored at 4C
RLU
100000
10000
1000
100
10
1
0
5
10
15
20
25
Week
200 mIU/ml
8 mIU/ml
0.5 mIU/ml
0 mIU/ml


Stability of Detection Antibody Stored at Room Temperature
RLU
100000
10000
1000
100
10
1
0
5
10
15
20
25
Week
200 mIU/mL
8 mIU/mL
0.5 mIU/mL
0 mIU/mL

Luteinizing Hormone Assay Validation Report

Theranos, Inc.

July 22, 2010

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1. Analyte Background

Luteinizing hormone (LH) is a member of the glycoprotein hormone family with a molecular weight of approximately 30,000 Daltons. Glycoprotein hormones are heterodimers consisting of a common alpha subunit and a unique beta subunit which confers biological specificity. Luteinizing hormone is expressed in the anterior pituitary gland and promotes spermatogenesis and ovulation by stimulating the testes and ovaries to synthesize steroids. LH is elevated during the luteal phase of menstrual cycle, primary hypogonadism, gonadotropin-secreting pituitary tumors and menopause.
The normal range of luteinizing hormone from literature:

FEMALES:	mIU/mL
Follicular phase	5.0 - 57
Ovulation phase	76 - 90
Luteal phase	3.0 - 41
Postmenopause	40 - 104
MALES:	2.5-19

2. Theranos Assay Specifications

The Theranos assay for Luteinizing hormone is a sandwich ELISA, specific for native human LH. Even though LH shares a common alpha subunit with other glycoprotein hormones like hCG and FSH, the Theranos assay is specific for LH. The Theranos LH assay is designed to detect LH in human whole blood, plasma and serum.
The upper limit of quantification (ULOQ) of the assay is 20 ng/mL and the lower limit of quantification (LLOQ) is 0.5 ng/mL. This range corresponds to 750 to 1 mIU/mL as verified on BioQuant reference ELISA mentioned below.

3. Reference Assays

1. Genway: cat# 40-056-205015

This is a solid phase ELISA configured for microtiter plates. The assay range is 200 – 10 mIU/mL using 25 µL samples. Assay time is approximately 1 hour. This kit has been used in-house to validate the Theranos assay. The protocol can be found at the following link.
http://www.genwaybio.com/images/gw_tds/elisa_kits/40-056-205015.pdf

2. BioQuant kit: Cat# BQ049F

This LH ELISA kit is used for the quantitative measurement of LH in human serum or plasma. It uses a solid phase direct sandwich method. The assay range is 50 – 3.1 mIU/mL using a 50 uL sample volume. The assay time is approximately 40 minutes. This kit has been used in-house to validate the Theranos assay. The protocol can be found at the following link.

http://www.bqkits.com/shopcart/images/product/BQ%20049F%20-%20LH.pdf

4. Cross Reactivity

Cross reactivity with estradiol, intact hCG, FSH, and progesterone analyte was evaluated to make sure that the chosen antibody pair is specific for LH. Cross reactivity was determined by testing each of the above mentioned analytes (at appropriate assay ranges) independently with the finalized LH antibody pair in the absence of LH. Results show that Theranos LH assay does not cross react with the tested analytes since even at the highest levels of potential cross-reactants the LH assay response corresponds to less than background.

Test Substance	Substance level	% Cross Reactivity
FSH	200 mIU/mL	0.0
Estradiol	32.4 ng/mL	0.0
intact hCG	90 ng/mL	0.0
Progesterone	200 ng/mL	0.0

5. Interfering Substances

Interference of estradiol, human chorionic gonadotropin (hCG), and follicle stimulating hormone (FSH) on the Theranos LH assay was evaluated. This was done by adding the above mentioned analytes at 3x their respective highest concentrations seen in clinical conditions to the LH standard curve. The levels tested were: estradiol 97,200 pg/mL, FSH 600 mIU/mL, and hCG 270 ng/mL. As shown below this assay is specific for luteinizing hormone.

Sample	Nominal [LH] ng/mL in sample	Control : LH Analyte only Mean RLU	Cal [LH] ng/mL in sample	Conc. % Recovery
1	20	237228	20.0	100
2	2	26451	2.0	100
3	0.5	7198	0.5	102
4	0	354	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH analyte + spiked Estradiol @ 97200 pg/mL Mean RLU	Cal [LH] ng/mL in sample	Conc. % Recovery
1	20 + spiked Estradiol	237008	20.0	100
2	2 + spiked Estradiol	27421	2.1	104
3	0.5 + spiked Estradiol	7862	0.6	113
4	0 + spiked Estradiol	332	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH analyte + spiked FSH @ 600 mIU/mL Mean RLU	Cal [LH] ng/mL in sample	Conc. % Recovery
1	20 + spiked FSH	209568	17.5	87
2	2 + spiked FSH	24459	1.8	92
3	0.5 + spiked FSH	6671	0.5	94
4	0 + spiked FSH	377	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH analyte + spiked intact hCG @ 270ng/mL Mean RLU	Cal [LH] ng/mL in sample	Conc. % Recovery
1	20 + spiked intact hCG	215854	18.0	90
2	2 + spiked intact hCG	25775	1.9	97
3	0.5 + spiked intact hCG	7102	0.5	101
4	0 + spiked intact hCG	449	0.0	NA

6. Precision Across Multiple instruments

A mid range LH calibrator concentration (5 ng/mL) was measured on 40 cartridges using 40 different instruments to determine the mid-range total system % CV.
CV% (any cartridge, any instrument) at mid range: 8.6 %

7. Precision Across Three Reagent Lots

A 12 point assay buffer standard curve was run on replicate cartridges (N = 6) across three reagent lots to determine precision. The inter lot % CV for concentration is 4%.

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%CV for Concentration

Inter Lot %CV							
Sample	**Nominal [LH] ng/mL in sample**	**Cal [LH] ng/mL in sample**			**Mean Cal [LH] ng/mL in sample**	**Stdev**	**%CV**
		Lot 1	**Lot 2**	**Lot 3**			
1	40	39.96	43.86	44.17	42.66	2.35	6
2	20	21.19	18.56	18.95	19.57	1.42	7
3	10	9.17	9.03	8.92	9.04	0.12	1
4	5	4.68	4.83	4.75	4.75	0.07	2
5	2	1.96	2.04	2.09	2.03	0.07	3
6	1	1.06	1.11	1.05	1.07	0.03	3
7	0.5	0.50	0.53	0.54	0.52	0.02	3
8	0.1	0.10	0.08	0.09	0.09	0.01	9
9	0	OORL	OORL	OORL	OORL	OORL	NA
						Avg % CV	**4**

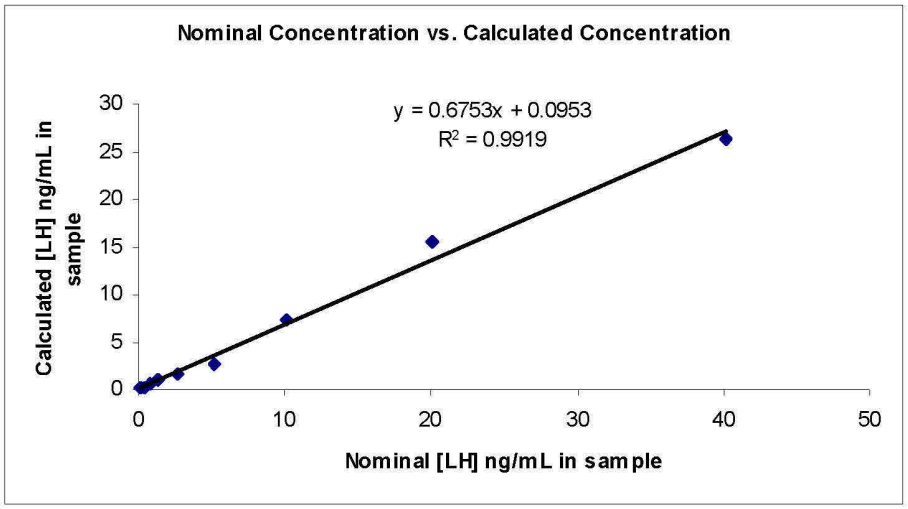
OORL = out of range low

8. Dilution Linearity in Blood

LH was spiked at 40 ng/mL into a blood sample with known endogenous concentration (0.14 ng/mL) and serially diluted with the same sample (unspiked) to generate a 9-point curve ranging from 40 - 0.14 ng/mL. The samples were analyzed on the Theranos system. Nominal concentration is defined as spiked plus endogenous level. Calculated concentration was back calculated using an assay buffer standard curve.

As seen below, recovery based on buffer calibration was consistent over the range averaging about 68 %. This indicates a sample matrix effect due to interactions of the analyte with plasma and red blood cells.

Nominal [LH] ng/mL in sample	Avg RLU	Stdev	CV%	Calc. [LH] ng/mL in sample	% recovery
40.14	395273	59689	15	26.28	65
20.14	236607	15929	7	15.56	77
10.14	113476	31509	28	7.39	73
5.14	41991	10417	25	2.71	53
2.64	26204	3199	12	1.68	64
1.39	14884	2384	16	0.95	68
0.77	9020	1704	19	0.56	74
0.45	4133	873	21	0.25	55
0.14	2484	303	12	0.14	100



9. Whole Blood Spike Recovery

Whole blood spike recovery experiment was conducted in samples from three subjects to see if there was variability in recovery. Average percentage recovery was 71%. Plasma recovered from spiked whole blood, however, gave a higher recovery as compared to the nominal in whole blood. Plasma gave recovery at roughly 134% relative to whole blood values. To determine the hematocrit effect, calculated LH concentrations from spiked whole blood were graphed against calculated LH concentration from plasma recovered from spiked whole blood. The average slope for blood versus plasma was 2.

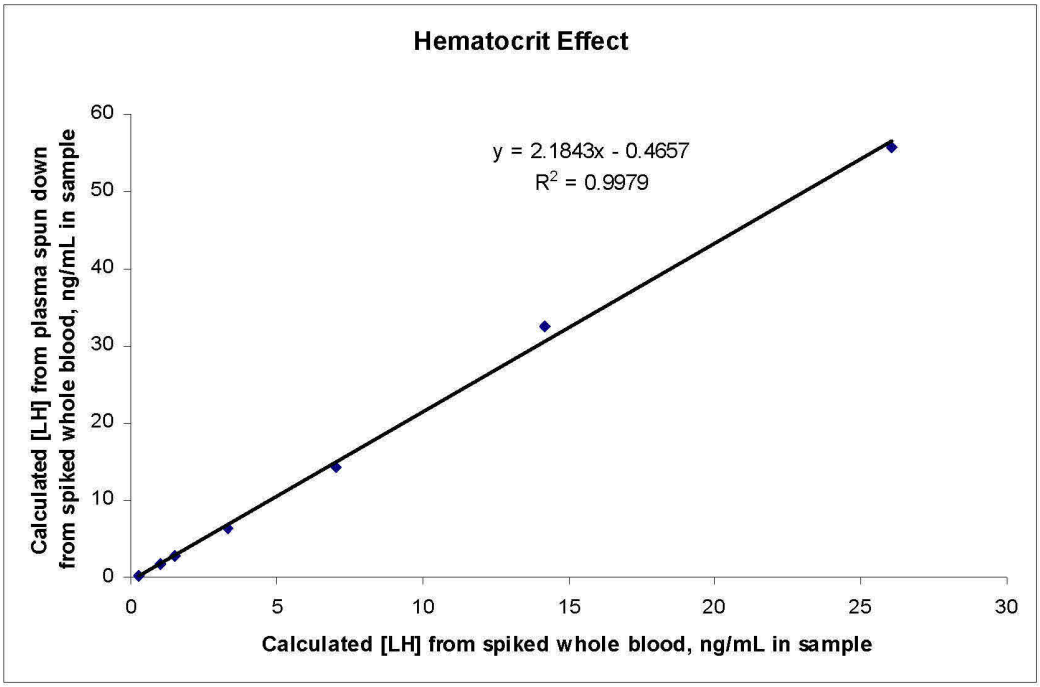
a. Sample # 1

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.28	392910	9722	2	26.08	65
2	20.28	218297	8904	4	14.2	70
3	10.28	108510	6125	6	7.02	68
4	5.28	50231	7052	14	3.31	63
5	2.28	21892	1903	9	1.52	67
6	1.28	13414	1345	10	0.99	77
7	0.28	2045	99	5	0.28	NA

Plasma from Spiked Whole Blood

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.28	795457	16525	2	55.64	138
2	20.28	484420	78603	16	32.54	160
3	10.28	219787	10241	5	14.3	139
4	5.28	97048	4976	5	6.29	119
5	2.28	40676	627	2	2.7	118
6	1.28	23592	865	4	1.63	127
7	0.28	2072	272	13	0.28	NA

Note: Nominal concentration is spiked concentration plus calculated endogenous level.



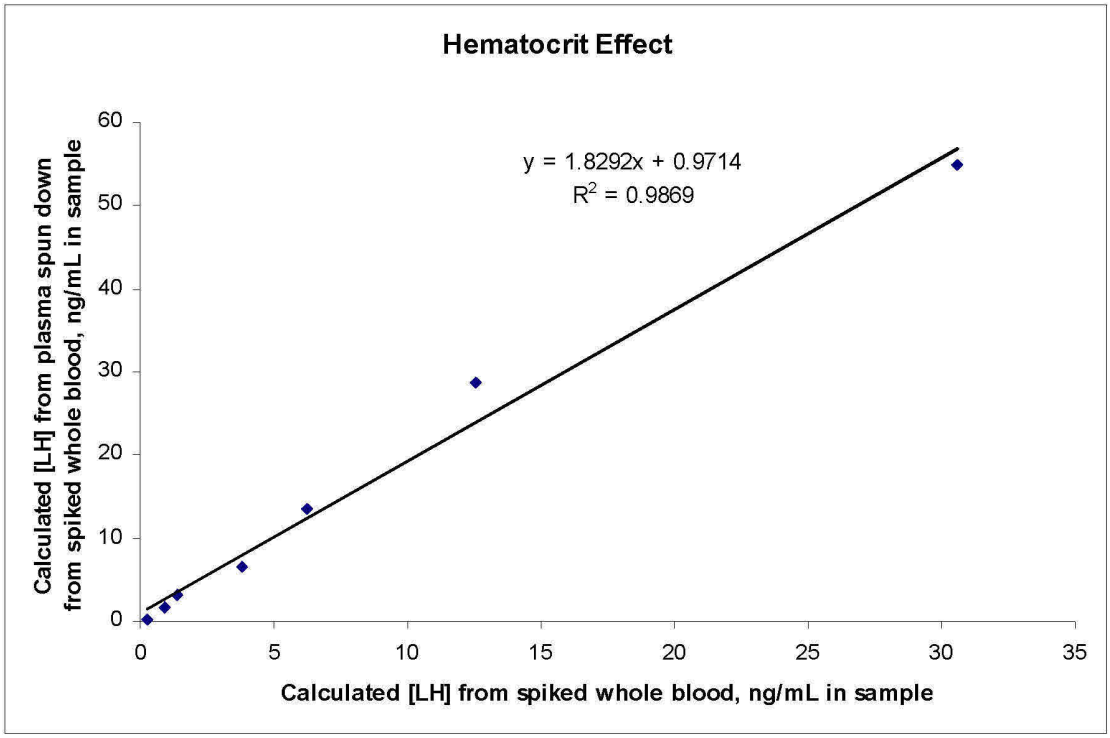
b. Sample # 2

Whole Blood Spike Recovery						
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.24	456998	88440	19	30.59	76
2	20.24	193586	16596	9	12.57	62
3	10.24	97001	5668	6	6.28	61
4	5.24	57953	6348	11	3.79	72
5	2.24	19773	4481	23	1.39	62
6	1.24	12493	1453	12	0.93	75
7	0.24	1387	80	6	0.24	100

Plasma from Spiked Whole Blood

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.3	787033	33051	4	54.99	136
2	20.3	429549	25799	6	28.65	141
3	10.3	209075	12491	6	13.59	132
4	5.3	101721	5735	6	6.59	124
5	2.3	47481	3612	8	3.13	136
6	1.3	23103	1588	7	1.6	122
7	0.3	2455	341	14	0.3	100

Note: Nominal concentration is spiked concentration plus calculated endogenous level.

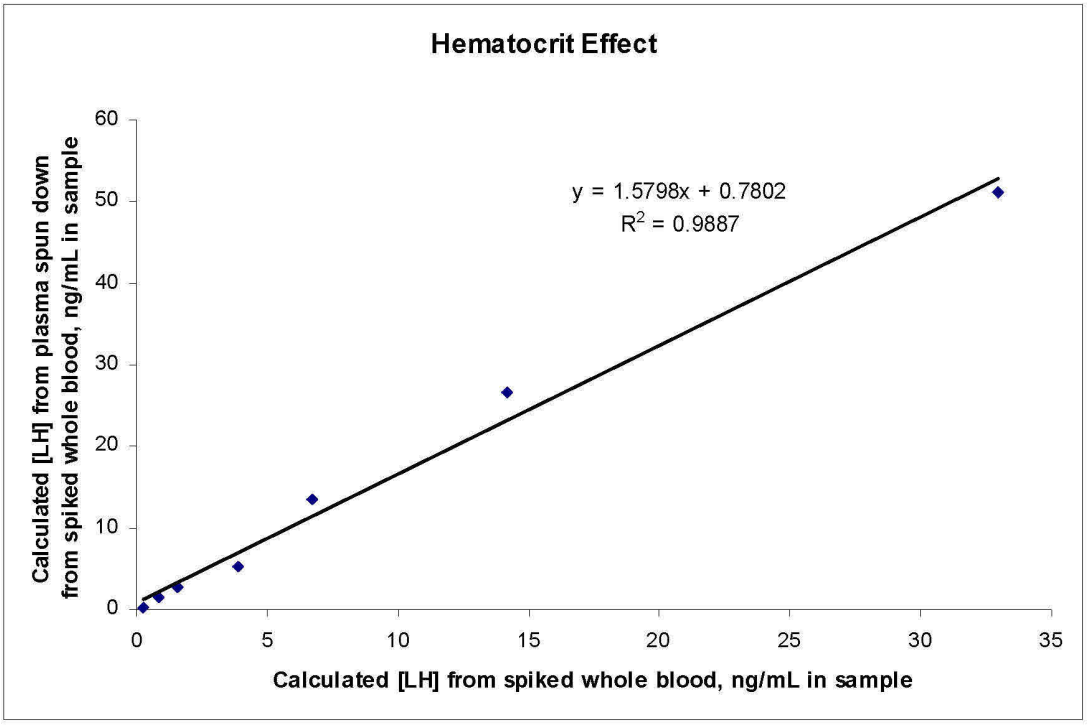


c. Sample # 3

Whole Blood Spike Recovery						
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.26	490119	29904	6	32.94	82
2	20.26	217443	21225	10	14.15	70
3	10.26	104291	14073	13	6.75	66
4	5.26	59499	4329	7	3.89	74
5	2.26	23354	1103	5	1.61	71
6	1.26	11222	702	6	0.85	68
7	0.26	1769	167	9	0.26	100

Plasma from Spiked Whole Blood						
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.31	736798	87422	12	51.14	127
2	20.31	401023	46292	12	26.65	131
3	10.31	207381	5354	3	13.48	131
4	5.31	81802	27389	33	5.31	100
5	2.31	39939	1792	4	2.65	115
6	1.31	20341	2655	13	1.42	108
7	0.31	2592	307	12	0.31	100

Note: Nominal concentration is spiked concentration plus calculated endogenous level.



10. Plasma Spike Recovery

Plasma samples with low endogenous LH levels were used for the spike recovery test. The spike recovery was tested for four samples to see if there was variability in the percentage recovery in different samples. An assay buffer standard curve was used to back calculate concentrations. Recovery for spiked plasma was consistent across the assay calibration range for all samples and from sample to sample averaging 81%.

a. Sample # 1

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Cal. [LH] ng/mL in sample	Percentage Recovery (%)
1	40.14	796915	52820	7	39.05	97
2	20.14	368468	92836	25	15.38	76
3	10.14	215261	28462	13	7.95	78
4	5.14	102764	12817	12	3.65	71
5	2.14	42875	4624	11	1.54	72
6	1.14	24802	3427	14	0.9	79
7	0.64	14249	1825	13	0.52	82
8	0.24	4722	756	16	0.18	74
9	0.14	3653	323	9	0.14	NA

Note: Nominal concentration is spiked concentration plus calculated endogenous level.

b. Sample # 2

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Cal. [LH] ng/mL in sample	Percentage Recovery (%)
1	40.31	495858	26228	5	33.35	83
2	20.31	273385	34310	13	17.89	88
3	10.31	141245	14248	10	9.14	89
4	5.31	69138	13228	19	4.5	85
5	2.31	30769	4044	13	2.08	90
6	1.31	15792	822	5	1.14	87
7	0.31	2627	154	6	0.31	100

Note: Nominal concentration is spiked concentration plus calculated endogenous level.

c. Sample # 3

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Cal. [LH] ng/mL in sample	Percentage Recovery (%)
1	40.31	491749	104981	21	33.06	82
2	20.31	267355	34330	13	17.48	86
3	10.31	130952	15873	12	8.47	82
4	5.31	52320	5724	11	3.44	65
5	2.31	29051	3785	13	1.97	85
6	1.31	14849	562	4	1.08	82
7	0.31	2525	179	7	0.31	100

Note: Nominal concentration is spiked concentration plus calculated endogenous level.

d. Sample # 4

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Cal. [LH] ng/mL in sample	Percentage Recovery (%)
1	40.28	413215	28659	7	27.5	68
2	20.28	265789	7265	3	17.38	86
3	10.28	113828	14550	13	7.37	72
4	5.28	66616	12631	19	4.34	82
5	2.28	27398	1576	6	1.87	82
6	1.28	12739	688	5	0.95	74
7	0.28	2096	347	17	0.28	100

Note: Nominal concentration is spiked concentration plus calculated endogenous level.

11. Matrix Effects

LH was spiked into lipemic plasma and hemolyzed whole blood. The purpose of the test was to determine the impact of the matrix on the assay response. The data generated from running hemolyzed whole blood and lipemic plasma were then compared to an assay buffer standard curve. The assay buffer standard curve's calibration equation was used to back calculate the spike recovery.
Conclusion: The average % recovery of hemolyzed and lipemic samples was 79 % and 77 % respectively. This is similar to the % recovery seen in normal plasma indicating minimal impact of these potential interfering factors

Hemolyzed Spiked Recovery

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	40.33	504860	60535	12	34	84
2	20.33	277517	54472	20	18.17	89
3	10.33	115848	17536	15	7.5	73
4	5.33	51858	14463	28	3.41	64
5	2.33	26914	5025	19	1.83	79
6	1.33	16085	744	5	1.16	87
7	0.33	2850	103	4	0.33	100

Lipemic Spiked Recovery

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	40.36	522644	36346	7	35.28	87
2	20.36	245357	14543	6	16.01	79
3	10.36	117576	7795	7	7.61	73
4	5.36	56034	2320	4	3.67	69
5	2.36	24601	1034	4	1.69	72
6	1.36	15738	808	5	1.13	83
7	0.36	3331	114	3	0.36	100

12. Determination of LLOQ and ULOQ

Lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were generated from 30 instruments, N = 3 cartridges for each LH standard. Calibrations are analyzed by our in house software suite adhering to FDA guidelines for assay calibration and LLOQ determination. Concentration CVs was determined using the back-calculated LH concentration. Theranos LH assay has the following LLOQ = 0.5 ng/mL and ULOQ = 20 ng/mL

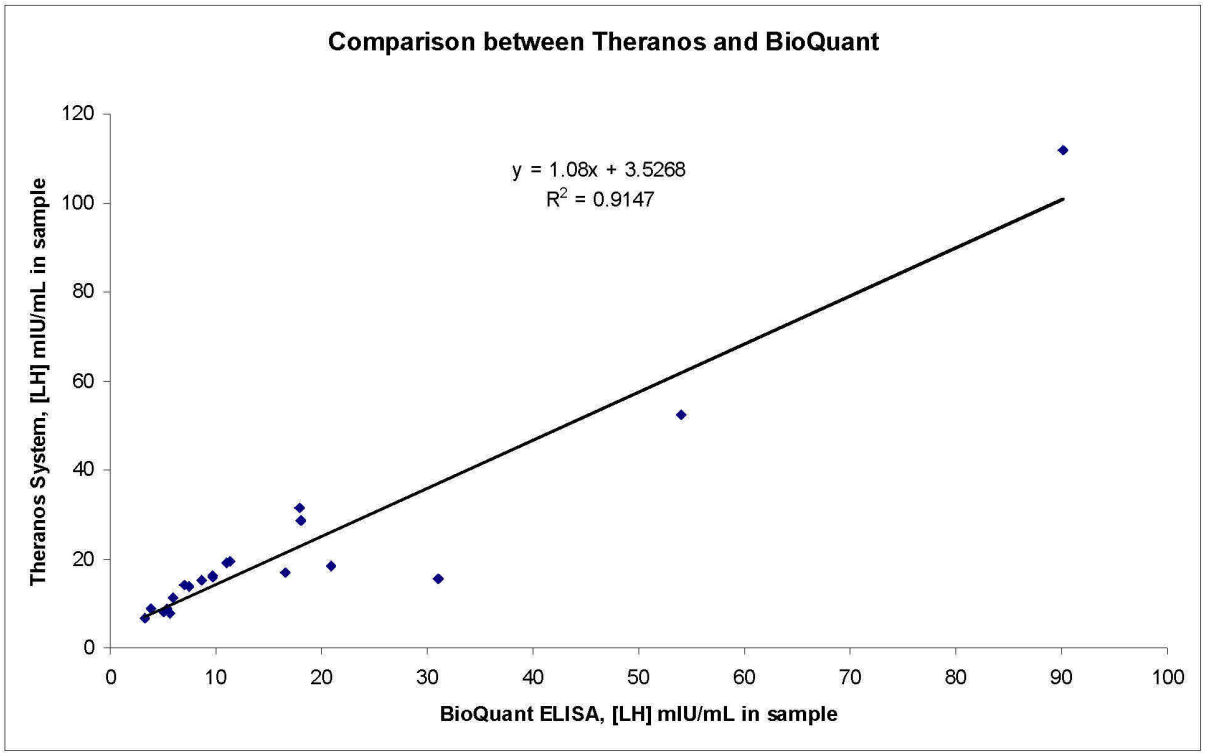
Sample	Nominal [LH] ng/mL in sample	MeanValue RLU	Std.Dev.	Signal CV%	Cal [LH] ng/mL in sample	Stdev	Conc. CV%	% Recovery of Conc.
1	40	560594	35754	6	40.02	2.76	7	100
2	20	303152	23025	8	20.25	1.59	8	101
3	10	146535	9487	6	9.53	0.70	7	95
4	5	79565	6465	8	5.10	0.38	7	102
5	2	31022	3203	10	1.96	0.19	10	98
6	1	15886	1079	7	1.01	0.01	1	101
7	0.5	8300	1007	12	0.55	0.06	11	109
8	0.1	2315	144	6	0.15	0.04	25	149
9	0	503	42	8	0.06	0.00	5	NA

13. Validation in Clinical Samples

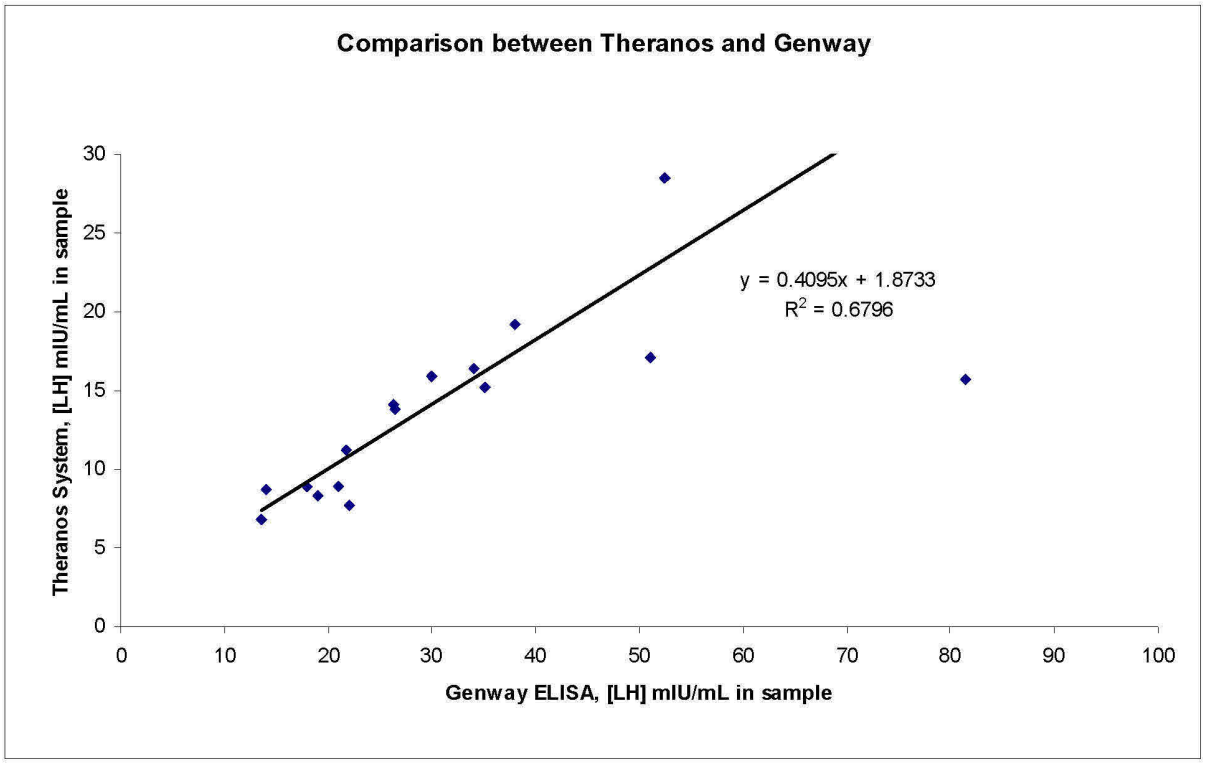
A total of thirty-five samples were run on the Theranos system and commercial Elisa kits (BioQuant & Genway). Correlation of results with those of the Bioquant kit was good but with the Genway kit was poor. However, the Genway and Bioquant correlation was also poor.

Note: The Theranos LH assay is specific for LH and does not show any cross reactivity with hCG(which is similar in structure). However the commercial LH ELISA kits tested showed high levels of cross reactivity (data not shown) with hCG. This explains why the pregnancy samples are detected as OORL in the Theranos assay whereas the kit data shows out of range high levels for those same samples.

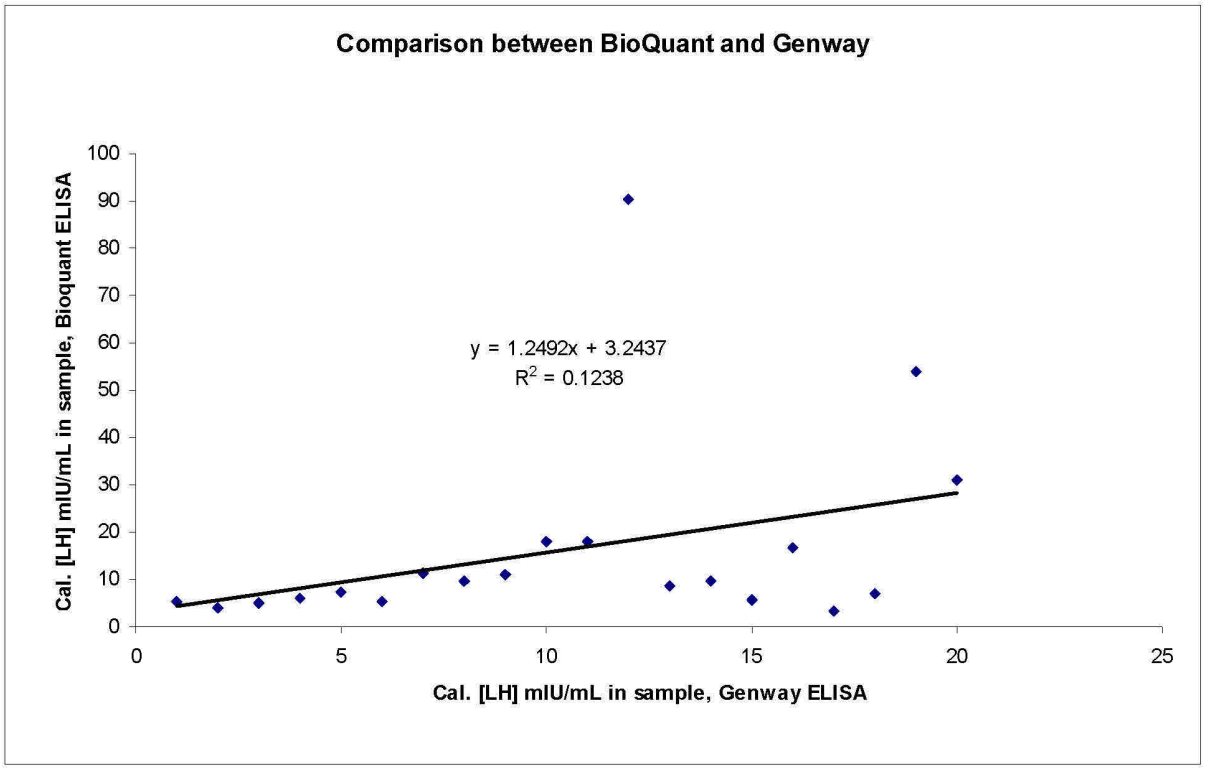
The following graph shows the correlation between Bioquant ELISA kit versus Theranos system for menstrual and post menstrual samples.



The following graph shows the correlation between Genway ELISA kit versus Theranos System for menstrual and post menstrual samples.



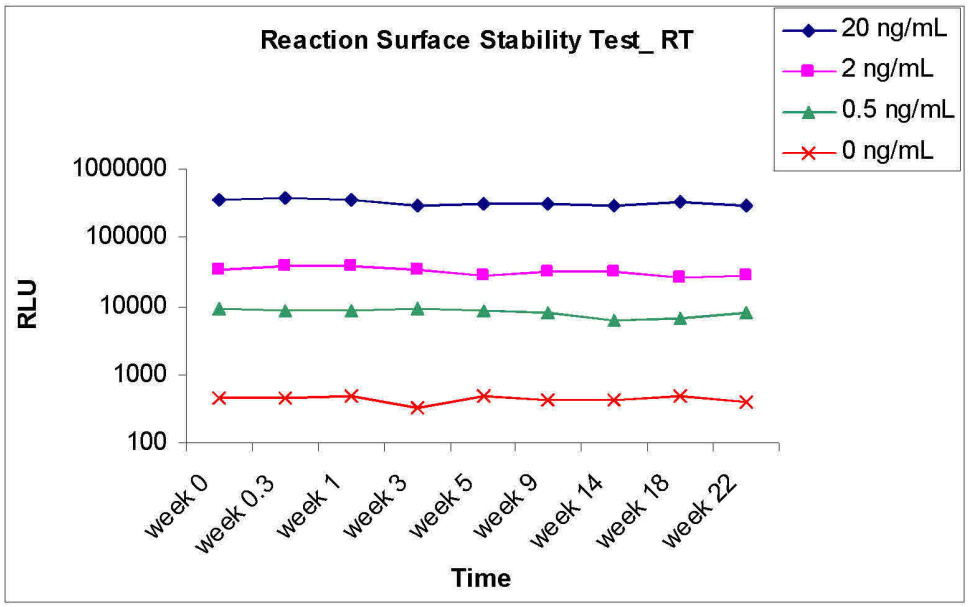
The following graph shows the correlation between Genway ELISA kit and BioQuant ELISA kit for menstrual and post menstrual samples.

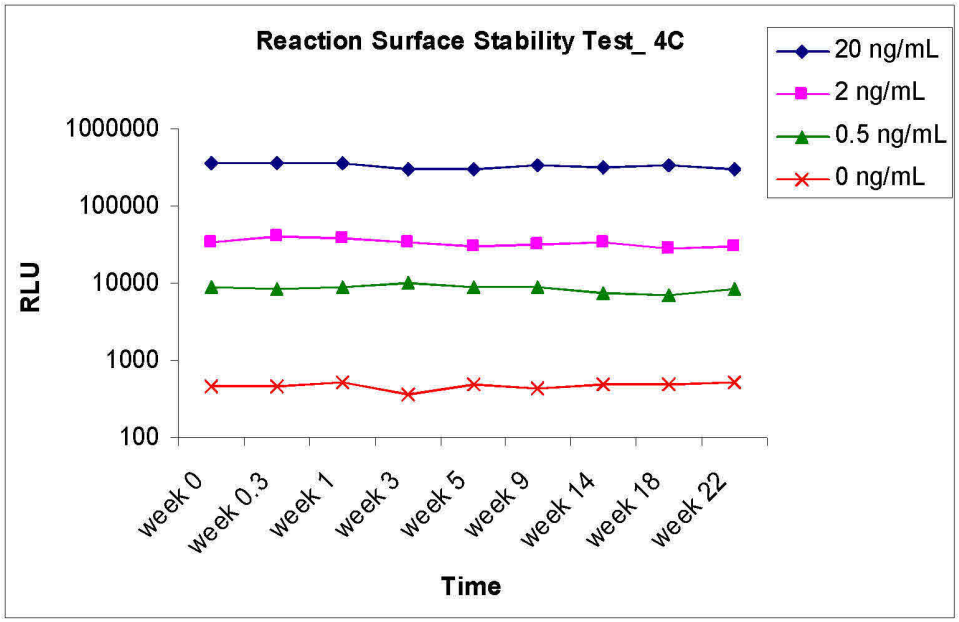


14. Stability of key reagents

a. Reaction Surface Stability

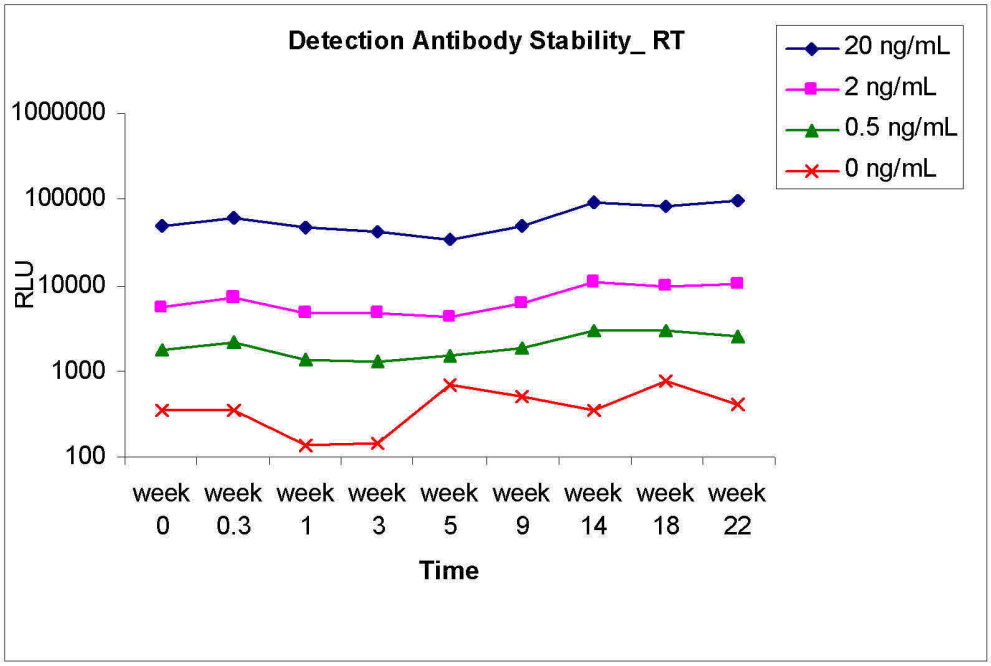
A 24 weeks stability test was set up for the reaction surface and evaluated on Theranos system. The reaction surface stability was tested on four different LH levels: 20, 2, 0.5, and 0 ng/mL. The stability of the reaction surface is tested at two temperature conditions, 4ºC and room temperature. Analyte standards were pre-made for the entire study, aliquoted and flash frozen for single time use.

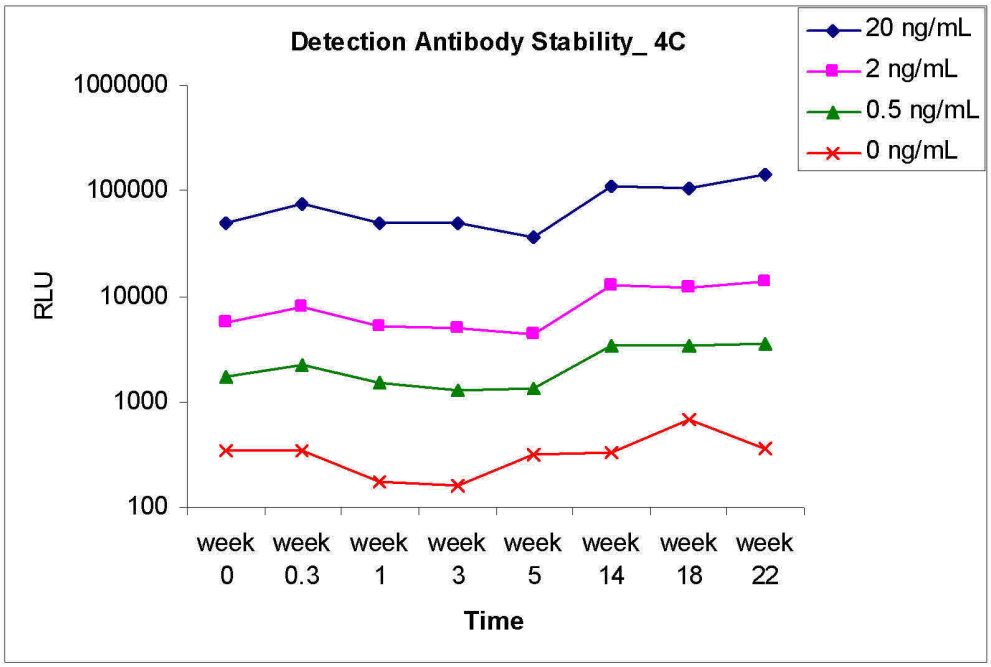

Reaction Surface Stability Test_ RT
20 ng/mL
2 ng/mL
0.5 ng/mL
0 ng/mL
RLU
1000000
100000
10000
1000
100
week 0
week 0.3
week 1
week 3
week 5
week 9
week 14
week 18
week 22
Time

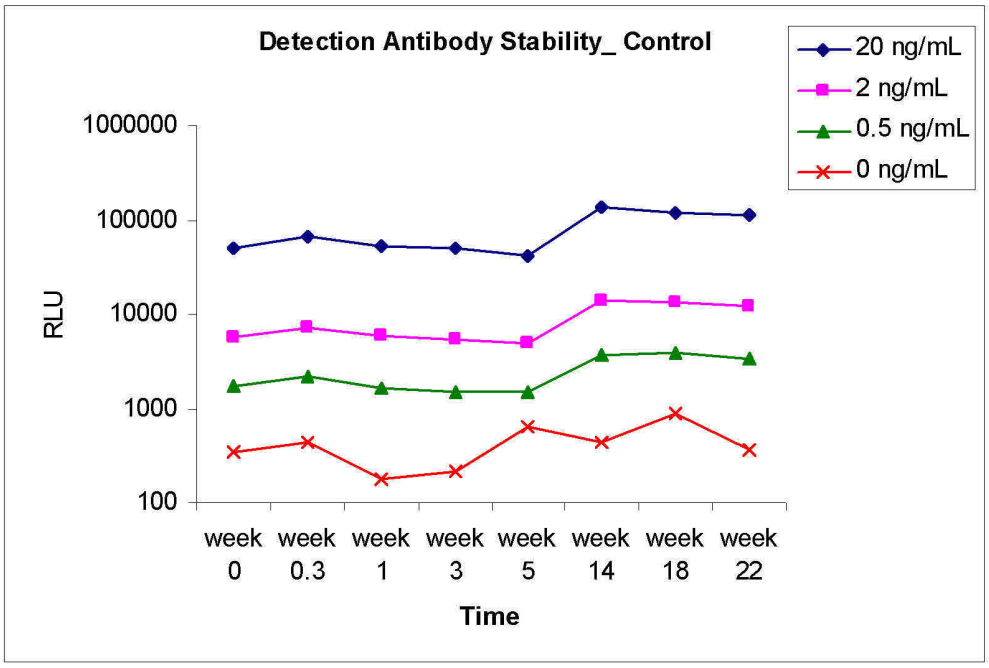

Reaction Surface Stability Test_ 4C
20 ng/mL
2 ng/mL
0.5 ng/mL
0 ng/mL
RLU
1000000
100000
10000
1000
100
week 0
week 0.3
week 1
week 3
week 5
week 9
week 14
week 18
week 22
Time

b. Detection Antibody Stability

Detection antibody stability at working concentration was tested for storage at 4°C and room temperature in an appropriate alkaline phosphatase stabilizer, with a 4-point assay buffer curve. Analyte standards were pre-made for the entire study, aliquoted and flash frozen for single time use. Data for 22 weeks are shown below.







Both the reaction surfaces and detection antibody at working concentration are stable for at least 6 months at both 4°C and room temperature.

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Estradiol (17 beta-Estradiol) Assay Validation Report

Theranos, Inc.

July 22, 2010

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1. Analyte 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 1-2 days before ovulation and then falls as LH levels peak (1).

In the blood, estradiol is bound with high affinity to Steroid Hormone Binding Globulin (SHBG) a homodimeric glycoprotein with an internal hydrophobic domain (2). 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 (2). Estradiol also associates with low affinity to other plasma proteins such as Human Serum Albumin (HSA) (3).

In post menopausal women, a high estradiol concentration is predictive of preserved bone mass and reduced fracture rates (4). Estradiol reduces bone resorption by inducing apoptosis of osteoclasts and is therefore protective against osteoporosis (5). Estradiol may also assist in attenuating the progression of diabetic kidney disease primarily by acting on TGF-beta-1 (6,7).

The normal range of estradiol:

Normal Levels	pg/mL
FEMALE	
Follicular phase	30-60
Preovulatory peak	109-408
Luteal phase	19-163
Postmenupausal	<50
MALES	13-54

2. Assay Specifications

The Theranos estradiol assay is designed to detect estradiol in human whole blood, plasma and serum. The assay has a reportable range of 35 – 500 pg/mL.

3. Reference Assays and Standards

There are a large number of commercially available ELISA kits for estradiol. The accuracy and precision of these assays varies widely, and many of them require extensive sample pre-treatment to extract estradiol from the biological matrix. Literature reports indicate that efforts to standardize measurement of estradiol in serum are ongoing (8).

The following commercial ELISA kits have been used in house:

a. Invitrogen Estradiol (E2) ELISA Catalog# KAQ0621 (Serum, EDTA Plasma, Heparin Plasma). Range is 10 pg/mL to 935 pg/mL.

b. Alpco Ultrasensitive Estradiol ELISA Catalog# EIA-4399 (Serum or Plasma). Range is 1.5 pg/mL to 200 pg/mL.
c. Alpco Estradiol EIA Catalog# 11-ESTHU-E01 (Serum Only). Range is 20 - 3200 pg/ml.
d. Assay Designs Estradiol-17b EIA Kit Catalog # 900-174 (Serum and Plasma, requires sample extraction). Range is 15 pg/mL to 1000 pg/mL.
e. Genway Estradiol, 17b (E2), ELISA Kit Catalog# 40-056-205004 (Serum or Plasma). Range is 5 pg/mL to 935 pg/mL. Product appears to be the same as Invitrogen Catalog# KAQ0621

There is no NISBC WHO standard available for estradiol at this time. The Theranos system is calibrated using >98% pure commercially available 17β-estradiol.

4. Cross Reactivity

Cross reactivity with other steroids was tested on the Theranos System. Estradiol (E2) antibodies are expected to cross react to some extent with estrone (E1) and estriol (E3). All commercial estradiol kits report some level of cross reactivity with these other forms of estrogen. Since other steroids may interact with binding proteins in a serum matrix and confound the cross reactivity studies, calibration and testing was carried out in a Low BSA buffer (0.03% BSA in TBS) instead of a serum, plasma or whole blood matrix for this experiment only.

Results:

- Cross reactivity was comparable to cross-reactivity reported by commercial kits.

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

OORL: Out of range low

The levels of estrone and estriol in the blood are related to the estradiol levels. Normal estrone levels in males and non-pregnant females are reported as less than 50 pg/mL (10,11). Based on the percentage cross reactivity, the calculated maximum change in recovered concentration of estradiol due to cross reactivity with 50 pg/mL of estrone would be 1.6 pg/mL, which is below the LLOQ of the assay and would not create a measurable impact on estradiol recovery.

In non-pregnant or postmenopausal women and in men, estriol levels are in the low pg/mL range (12) while only in second and third trimester pregnancy do they rise into the low ng/mL range (13). The calculated maximum change in estradiol recovery due to 2 ng/mL of estriol is 1 pg/mL, also too small to create a measurable impact on estradiol recovery.

In conclusion, the levels of cross reactivity with E1 and E3 observed on the Theranos System are minimal and will not present significant error in measuring estradiol levels accurately in males or in non-pregnant, postmenopausal, or pregnant females.

5. Dilution Linearity

Dilution linearity in serum and plasma was tested by performing a 1:2 serial dilution of a high endogenous or estradiol spiked sample into a low endogenous sample of the same matrix and measuring the recovery over the range.

Results:

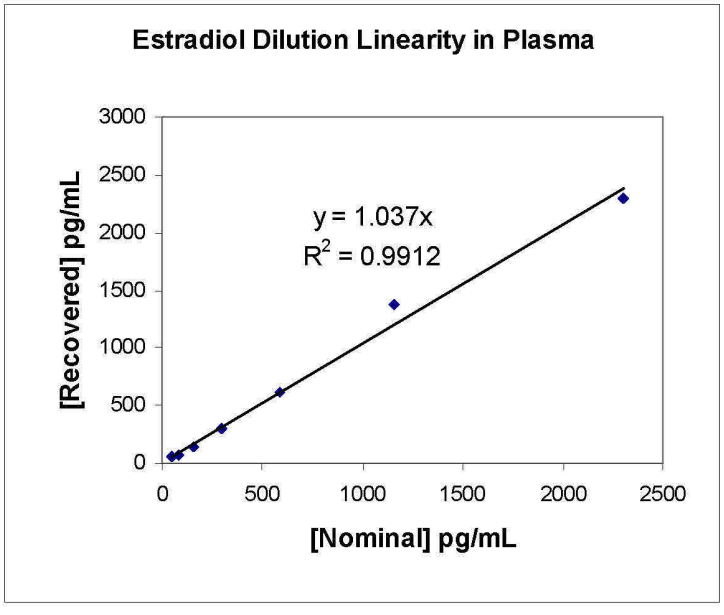
- Recovery was consistently within 25% of nominal over the range of the dilution series in serum and plasma.

a. Dilution Linearity in Plasma

Male plasma spiked with 2300 pg/mL estradiol was diluted into the low endogenous plasma.

[Nominal] pg/mL	Signal			Calculated Concentration			
	Mean RLU	StDev	CV%	Mean Conc pg/mL	StDev	CV%	% Recovery
2300	2038	72	4	2300	9	0	100
1157	2824	165	6	1446	128	9	125
586	5318	342	6	616	53	9	105
301	9152	456	5	296	22	7	98
158	14108	746	5	147	14	10	93
86	19545	1798	9	72	19	26	84
51	22634	2295	10	52	21	39	103
15	29443	1634	6	OORL	-	-	-

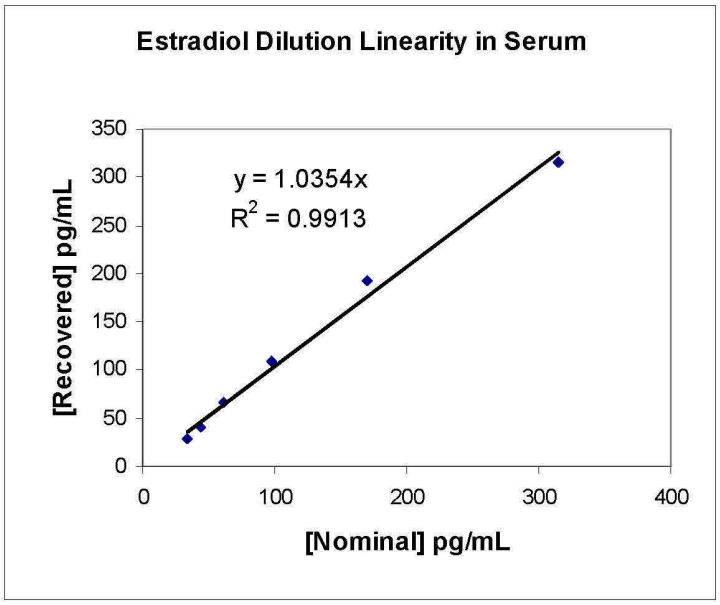
OORL: Out of range low



b. Dilution Linearity in Serum

Female serum from menstrual cycle day 16 was diluted into a low endogenous serum sample.

[Nominal] pg/mL	Signal			Calculated Concentration			
	Mean RLU	StDev	CV%	Mean Conc pg/mL	StDev	CV%	% Recovery
315	5822	5036	86	315	1	0	100
170	12026	993	8	193	36	19	113
98	15828	1247	8	109	16	15	112
61	18853	2391	13	66	12	18	108
43	23158	1198	5	42	8	19	96
34	25102	4765	19	29	-	-	86



6. Whole Blood Spike Recovery

Three samples of whole blood were spiked with estradiol and the samples were measured on the Theranos System.

Results:

- All samples showed recovery within 25% of nominal at both spiked levels, meeting the acceptance criteria for whole blood spike recovery.

Recovered [Estradiol] pg/mL in Spiked Whole Blood

Sample #	[Spiked] pg/mL	[Nominal] pg/mL	Recovered [Estradiol] pg/mL			
			Mean Conc.	StDev	CV%	% Recovery
1	800	938	996	134	13	106
	200	338	420	50	12	124
	0	138	138	65	47	-
2	800	874	657	158	24	75
	200	274	320	65	20	117
	0	74	74	25	34	-
3	800	893	885	164	18	99
	200	293	271	62	23	92
	0	93	93	2	2	-

7. Hematocrit Effect

The 3 whole blood samples spiked in part (6) were spun down and the recovery of the estradiol spike in the resulting plasma was measured in the Theranos System.

Results:

- All samples showed recovery within 25% of nominal at both spiked levels.

Recovered [Estradiol] pg/mL in Plasma From Spiked Whole Blood

Sample #	[Spiked] pg/mL	[Nominal] pg/mL	Recovered [Estradiol] pg/mL			
			Mean Conc.	StDev	CV%	% Recovery
1	800	938	949	-	-	101
	200	338	421	20	5	125
	0	138	137	34	25	99
2	800	874	1061	226	21	121
	200	274	293	43	15	107
	0	74	66	28	43	89
3	800	893	1011	167	17	113
	200	293	341	32	9	116
	0	93	79	12	16	85

8. Selectivity

Whole blood from 10 patients spiked at 0 (endogenous), 200 and 350 pg/mL was tested on the Theranos System.

Results:

- 8 out of 10 samples showed recovery within 25% of nominal at both spiked levels, meeting the acceptance criteria for selectivity.

Spiked Whole Blood, 10 Patients

				Recovered [Estradiol] pg/mL			
Sample #	Sex	Spike pg/mL	Nominal pg/mL	Mean Conc	StDev	CV %	% Recovery
1	M	0	36	36	2	5	-
		200	236	279	87	31	118
		350	386	302	39	13	78
2	M	0	21	21	3	14	
		200	221	227	38	17	103
		350	371	445	70	16	120
3	M	0	0	OORL	-	-	-
		200	200	200	37	19	100
		350	350	255	11	4	73
4	M	0	24	24	6	26	-
		200	224	203	42	21	91
		350	374	298	35	12	80
5	M	0	42	42	8	19	-
		200	242	268	104	39	111
		350	392	425	46	11	108
6	M	0	36	36	16	45	-
		200	236	266	25	9	113
		350	386	464	105	23	120
7	F	0	0	OORL	-	-	-
		200	200	197	45	23	99
		350	350	341	97	28	97
8	F	0	0	OORL	-	-	-
		200	200	178	18	10	89
		350	350	372	26	7	106
9	F	0	20	20	1	3	
		200	220	170	27	16	77
		350	370	372	15	4	100
10	F	0	34	34	5	15	
		200	234	168	30	18	72
		350	384	334	50	15	87

OORL: Out of range low

9. Extended Range

The assay was tested for high dose hook effect with estradiol levels above 10,000 pg/mL.

Results:

- The assay response is weak above 4000 pg/mL (only seen in third trimester pregnancy) but as expected for a competitive assay, no high dose hook effect was observed.

[Estradiol] pg/mL	Mean RLU	StDev	CV%
16200	1219	112	9
8100	1385	144	10
4056	1925	142	7
2031	2307	204	9
681	5672	547	10
231	12299	955	8
81	21294	260	1
31	25793	945	4
16	30241	1226	4
6	36938	2119	6

10. Determination of LLOQ and ULOQ

Lower Limit of Quantification (LLOQ) and Upper Limit of Quantification (ULOQ) are specified for the system. LLOQ and ULOQ will be verified for each lot of cartridges during calibration. Typical data used to calibrate one reagent lot is shown below. These data were generated from 36 instruments, N = 3 cartridges for each concentration. Calibrations are analyzed by our in house software suite adhering to FDA guidelines for assay calibration and LLOQ determination. Back-calculations on each cartridge determined the concentration CVs.

For this reagent lot, LLOQ = 21.8 pg/mL and ULOQ = 636.0 pg/mL meeting the system specifications.

Standard Curve – Serum Calibrators

	RLU			Back -Calculated Concentration			
[Estradiol] pg/mL	Mean RLU	StDev	CV %	Mean Conc pg/mL	StDev	CV %	% Recovery
636.0	2526	148	6	529	141	27	83
321.0	3081	160	5	303	35	12	94
163.5	4128	559	14	172	37	21	105
132.0	4803	758	16	141	26	18	107
100.5	6434	387	6	94	5	5	93
84.8	7404	457	6	79	4	5	93
69.0	8021	1141	14	69	13	18	100
53.3	9445	1403	15	57	9	16	108
37.5	12710	1265	10	35	5	15	93
21.8	16561	1268	8	21	3	14	96
12.3	18288	2134	12	19	2	10	157
6.0	20917	2620	13	OORL			

OORL: Out of range low

Validation in Clinical Samples

Clinical serum samples obtained from ProMedDx were run on the Theranos System and on commercial reference methods; Alpco Estradiol EIA Catalog # 110-ESTHU-E01, Alpco Ultrasensitive Estradiol ELISA Catalog # EIA-4399, and Invitrogen Estradiol ELISA Catalog # KAQ0621.

Results:

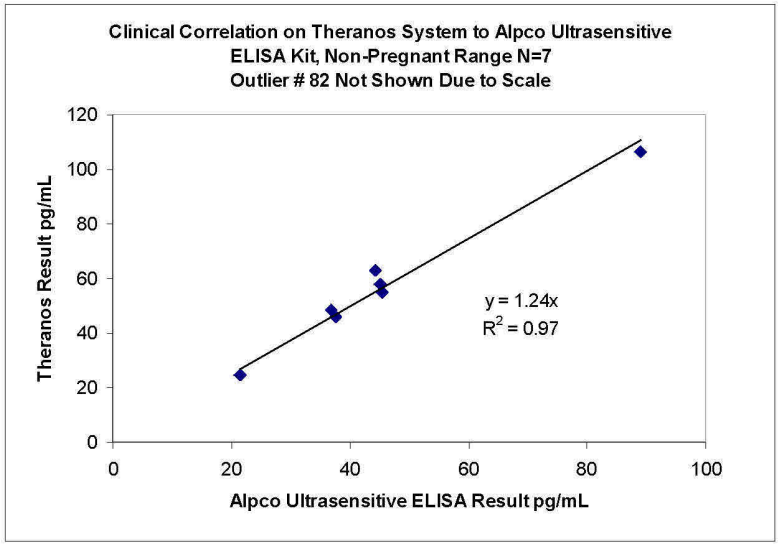
- Theranos System results correlated well with the commercial methods tested
- Correlation of the Theranos System was best with the Alpco Ultrasensitive ELISA with the exception of sample #82.
- In the non-pregnant range, correlation of Theranos results to each reference method was better than the correlation of the Invitrogen result to the Alpco result.

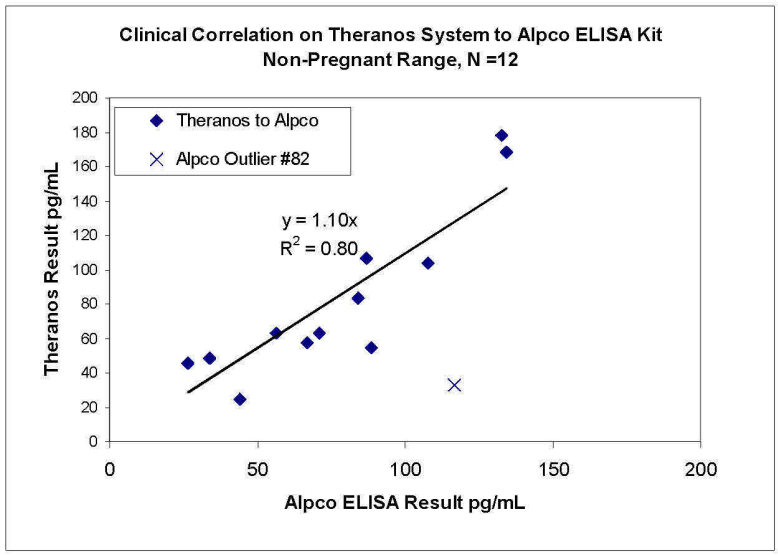
Note about sample #82: Postmenopausal women are expected to have < 50 pg/mL estradiol (14), so the Alpco Regular and Ultrasensitive Kit results appear to be discrepant, whereas Invitrogen and Theranos results match closely and are consistent with the expected levels of estradiol for postmenopausal women. These samples were obtained from women not receiving hormone replacement therapy.

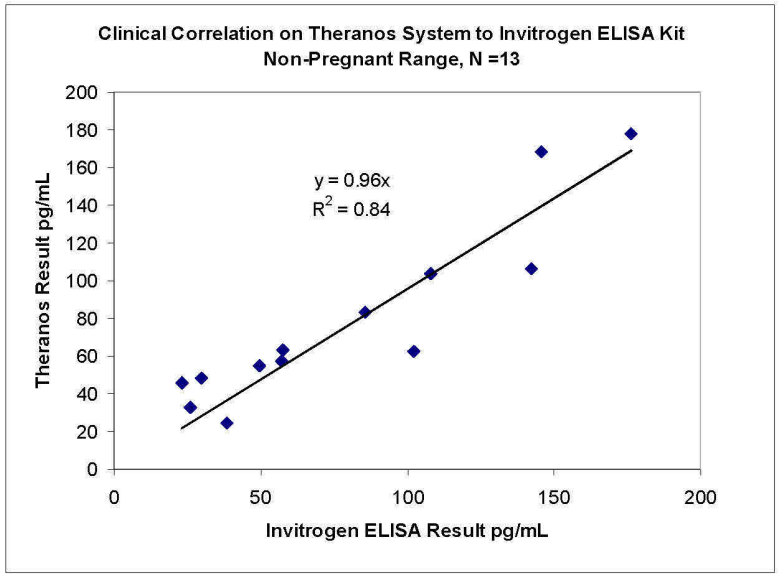
Clinical Sample Results

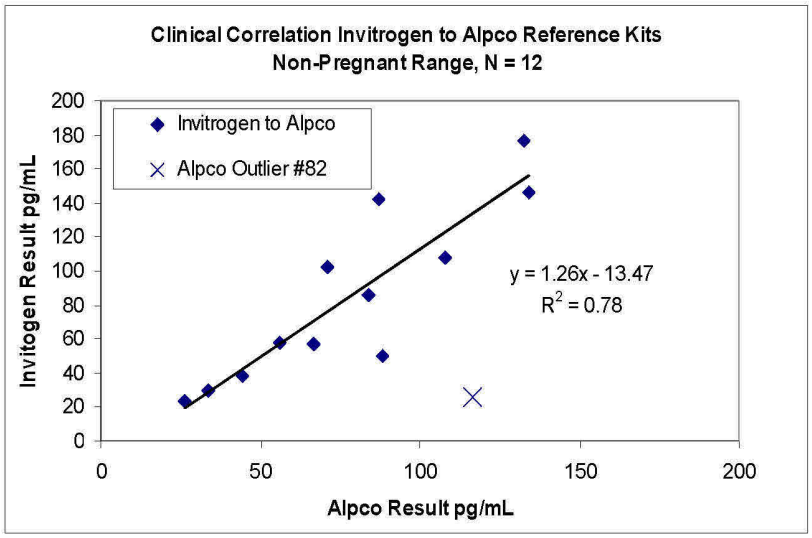
			Reference Result pg/mL			Theranos Result pg/mL		
Sample #	Set	Day/Week	Invitrogen	Alpco	Alpco Ultras.	Mean Conc.	StDev	CV %
81	Postmenopausal	N/A	38	44	22	25	6	25
82	Postmenopausal	N/A	26	117	283	33	6	18
1	Menstruation	1	57	67	45	58	12	21
2	Menstruation	2	30	34	37	48	1	2
5	Menstruation	5	23	26	38	46	6	13
8	Menstruation	8	50	88	46	55	10	17
9	Menstruation	9	102	71	44	63	7	11
12	Menstruation	12	86	84	NT	83	15	18
13	Menstruation	13	142	87	89	106	8	8
15	Menstruation	15	176	133	NT	178	34	19
21	Menstruation	21	146	134	NT	168	36	22
26	Menstruation	26	108	108	NT	104	4	4
28	Menstruation	28	58	56	NT	63	6	9
51	Pregnant	5	337	210	179	177	38	21
52	Pregnant	7	440	689	NT	OORH		
53	Pregnant	5	271	182	NT	139	11	8
55	Pregnant	9	333	242	NT	142	23	16

NT = Not tested OORH = Out of Range High


Clinical Correlation on Theranos System to Alpco Ultrasensitive
ELISA Kit, Non-Pregnant Range N=7
Outlier # 82 Not Shown Due to Scale
Theranos Result pg/mL
Alpco Ultrasensitive ELISA Result pg/mL
y = 1.24x
R2 = 0.97
0
20
40
60
80
100
120


Clinical Correlation on Theranos System to Alpco ELISA Kit
Non-Pregnant Range, N =12
Theranos to Alpco
Alpco Outlier #82
y = 1.10x
R2 = 0.80
Theranos Result pg/mL
Alpco ELISA Result pg/mL
0
20
40
60
80
100
120
140
160
180
200
50
150

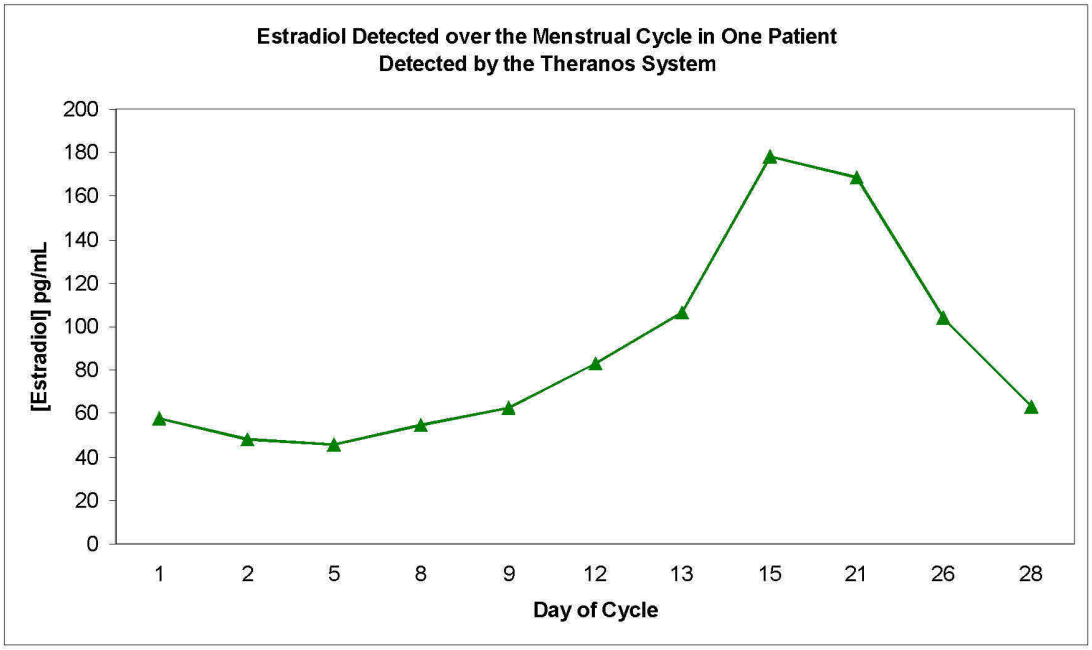

Clinical Correlation on Theranos System to Invitrogen ELISA Kit
Non-Pregnant Range, N =13
y = 0.96x
R2 = 0.84
Theranos Result pg/mL
Invitrogen ELISA Result pg/mL
0
20
40
60
80
100
120
140
160
180
200
50
150



As part of the assay clinical validation we measured the levels of estradiol over a menstrual cycle using serum samples from a single individual from ProMedDx.

Results:

- Estradiol levels measured on the Theranos System in a single patient over time are in accordance with expected estradiol trends during the menstrual cycle.



11. Stability

For each stability time point, all reagents are formulated fresh except the test reagent. In addition, a control is included with all reagents formulated fresh from stock materials.

Results:

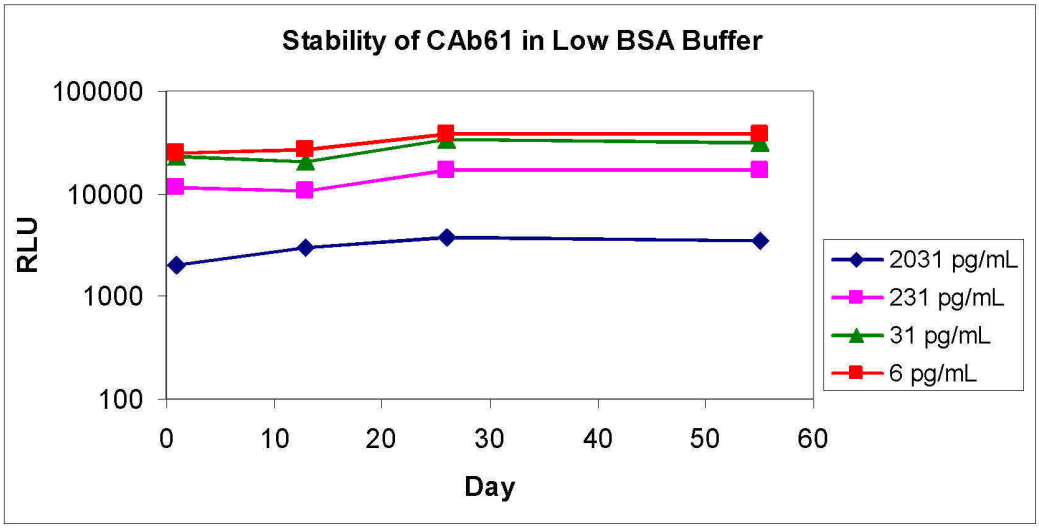
- In these initial tests, the reagents are showing good stability.

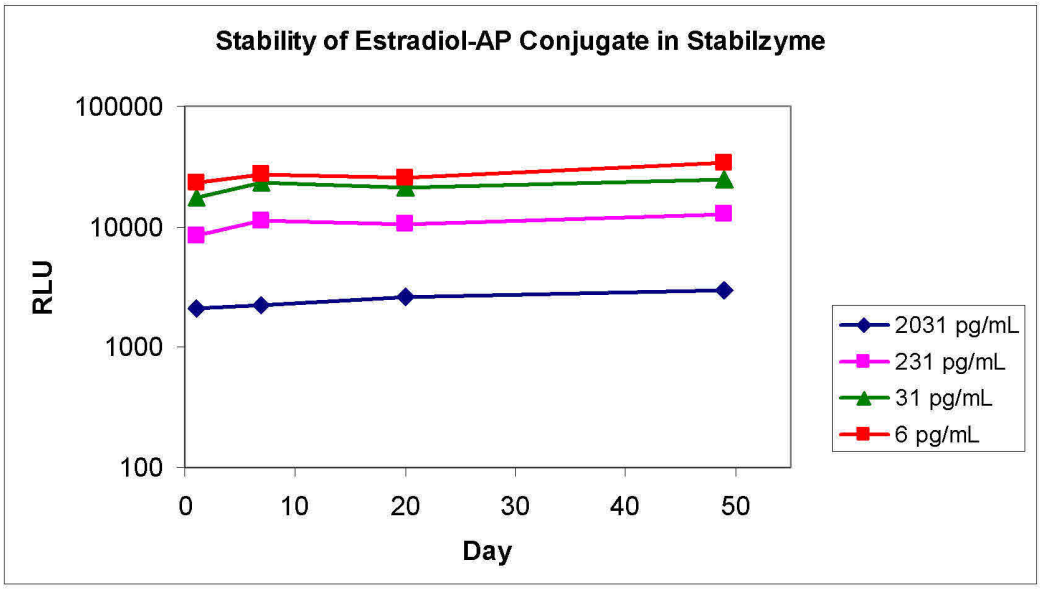
Capture Antibody Stability

			Control				Stored at 4°C			
Date	Day	[Estradiol] pg/mL	Mean RLU	StDev	CV%	Mod.	Mean RLU	StDev	CV%	Mod.
5/12/10	1	2031					2027	285	14	12.1
		231					11698	315	3	2.1
		31					22704	2166	10	1.1
		6					24625	2616	11	
5/25/10	13	2031	2829	119	4	9.1	2930	46	2	9.2
		231	10620	672	6	2.4	10635	399	4	2.5
		31	21209	1295	6	1.2	20568	1126	5	1.3
		6	25674	1000	4		27094	3242	12	
6/07/10	26	2031	3471	497	14	10.6	3776	365	10	9.9
		231	14658	768	5	2.5	17118	1896	11	2.2
		31	30742	64	0	1.2	33954	1399	4	1.1
		6	36691	2352	6		37385	732	2	
7/06/10	55	2031	3450	196	6	10.6	3530	236	7	10.7
		231	16061	1093	7	2.3	17119	902	5	2.2
		31	29822	2460	8	1.2	31552	449	1	1.2
		6	36518	4031	11		37943	950	3	

Stability of Estradiol-AP

			Control				Stored at 4°C			
Date	Day	[Estradiol] pg/mL	Mean	StDev	CV%	Mod.	Mean	StDev	CV%	Mod.
5/18/10	1	2031	2107	426	20	10.9				
		231	8567	1628	19	2.7				
		31	17443	3200	18	1.3				
		6	23070	3594	16					
5/25/10	7	2031	2829	119	4	9.1	2235	366	16	12.2
		231	10620	672	6	2.4	11370	1719	15	2.4
		31	21209	1295	6	1.2	23443	3894	17	1.2
		6	25674	1000	4		27354	3539	13	
6/7/10	20	2031	3471	497	14	10.6	2603			10.0
		231	14658	768	5	2.5	10618	682	6	2.4
		31	30742	64	0	1.2	21251	1680	8	1.2
		6	36691	2352	6		25983	74	0	
7/6/10	49	2031	3450	196	6	10.6	2940	55	2	11.5
		231	16061	1093	7	2.3	12781	358	3	2.7
		31	29822	2460	8	1.2	25014	2565	10	1.4
		6	36518	4031	11		33903	283	1	


Stability of CAb61 in Low BSA Buffer
100000
10000
1000
100
RLU
0
10
20
30
40
50
60
Day
2031 pg/mL
231 pg/mL
31 pg/mL
6 pg/mL


Stability of Estradiol-AP Conjugate in Stabilzyme
100000
10000
1000
100
RLU
0
10
20
30
40
50
Day
2031 pg/mL
231 pg/mL
31 pg/mL
6 pg/mL

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