Luteinizing Hormone Assay Validation Report

Theranos, Inc.

~~July~~ Novemeber 29~~22~~, 201~~1~~0

This Validation Report contains Theranos Confidential Information and is being provided to Celgene under the parties' Mutual Confidentiality Agreement. Celgene may provide this Report to B2S Consulting – specifically and only to Dr. Bowsher – under the parties' three-way Unilateral Disclosure Agreement for the limited purposes set forth therein. Any further dissemination, use or disclosure of the Report, in whole or in part, is strictly prohibited.

Theranos Internal Only

theranos®
redefining healthcare

TABLE OF CONTENTS

[TOC \o "1-3" \f \h \z]

Theranos Internal Only

theranos®
redefining healthcare

1. Analyte Background[TC " Analyte Background" \f C \l "1"]

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
Lutcal phasc	3.0 - 41
Postmenopause	40 - 104
MALES:	2.5-19

2. Theranos Assay Specifications
[TC "Theranos Assay Specifications" \f C \l "1"]

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~~ 410 to 1 mIU/mL as verified on BioQuant reference ELISA mentioned below.

3. Reference Assays[TC "Reference Assays" \f C \l "1"]

~~1.~~ Genway: cat# 40-056-205015

1.

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.
[HYPERLINK "http://www.genwaybio.com/images/gw_tds/clisa_kits/40-056-205015.pdf"]

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Tab after: 0.5" + Indent at: 0.5"

~~2.~~ BioQuant kit: Cat# BQ049F

2.

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.

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Tab after: 0.5" + Indent at: 0.5"

Formatted: Indent: Before: 0.25", First line: 0", Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

Theranos Confidential Only

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

Formatted: Indent: Before: 0.25", First line: 0", Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

4. Cross Reactivity[TC "Cross Reactivity" \f C \l "1"]

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[TC "Interfering Substances" \f C \l "1"]

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.

THERANOS CONFIDENTIAL

theranos®
redefining healthcare

Sample	Nominal [LH] ng/mL in sample	Control : LH Analyte Mean RLU	Cal [LH] ng/ml in sample	Conc. % Recovery
1	20	105440	20.0	100
2	2	11829	2.0	100
3	0.5	3152	0.5	100
4	0	232	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH + spiked Estradiol @ 97200 pg/ml Mean RLU	Cal [LH] ng/ml in sample	Conc. % Recovery
1	20 + spiked Estradiol	138449	27.1	136
2	2 + spiked Estradiol	16198	2.8	138
3	0.5 + spiked Estradiol	4180	0.7	135
4	0 + spiked Estradiol	269	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH + spiked FSH @ 600 mIU/ml Mean RLU	Cal [LH] ng/ml in sample	Conc. % Recovery
1	20 + spiked FSH	105232	20.0	100
2	2 + spiked FSH	11786	2.0	100
3	0.5 + spiked FSH	3174	0.5	101
4	0 + spiked FSH	320	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH + spiked intact hCG @ 270ng/ml Mean RLU	Cal [LH] ng/ml in sample	Conc. % Recovery
1	20 + spiked hCG	114470	21.9	110
2	2 + spiked hCG	13876	2.4	118
3	0.5 + spiked hCG	3540	0.6	113
4	0 + spiked hCG	269	0.0	NA

Sample	Nominal [LH] ng/mL in sample	LH + spiked Progesterone @ 600 ng/ml Mean RLU	Cal [LH] ng/ml in sample	% Recovery from control
1	20 + spiked Estradiol	94533	17.7	89
2	2 + spiked Estradiol	10876	1.8	92
3	0.5 + spiked Estradiol	2924	0.5	92
4	0 + spiked Estradiol	211	0.0	NA

THERANOS CONFIDENTIAL

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

Formatted: Indent: Before: 0.25"

6. Precision Across Multiple instruments[TC "Precision Across Multiple instruments " \f C \l "1"]

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 [TC "Precision Test Across Three Reagent Lots" \f C \l "1"]

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 410%.

%CV for Concentration							
Inter %CV							
Sample	[LH] mIU/mL	Lot 1	Lot 2	Lot 3	Mean	Stdev	Inter %CV
	in sample	Cal. [LH] mIU/mL	Cal. [LH] mIU/mL	Cal. [LH] mIU/mL	Cal. [LH] mIU/mL	3 lots	3 lots
1	627.00	618.63	654.01	681.11	651.25	31	5
2	347.50	325.24	353.05	311.55	329.95	21	6
3	167.30	173.73	170.63	133.84	159.40	22	14
4	77.74	83.53	95.04	92.18	90.25	6	7
5	41.28	43.66	37.28	41.25	40.73	3	8
6	19.55	17.82	18.61	19.91	18.78	1	6
7	14.95	13.73	15.45	16.42	15.20	1	9
8	11.60	10.74	9.05	13.64	11.14	2	21
9	7.99	8.89	8.75	8.35	8.66	0	3
10	3.62	4.37	3.19	3.18	3.58	1	19
11	1.23	1.24	1.46	OORL	1.35	0	12
12	0.00	OORL	OORL	OORL	OORL	NA	NA
					Average Inter %CV		10

%CV for Concentration							
Inter Lot %CV							
Sample	Nominal [LH] ng/mL	Cal [LH] ng/mL in sample			Mean Cal [LH]		
	in sample	Lot 1	Lot 2	Lot 3	ng/mL in sample	Stdev	%CV
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	4.69	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

Formatted: No bullets or numbering

Theranos Confidential

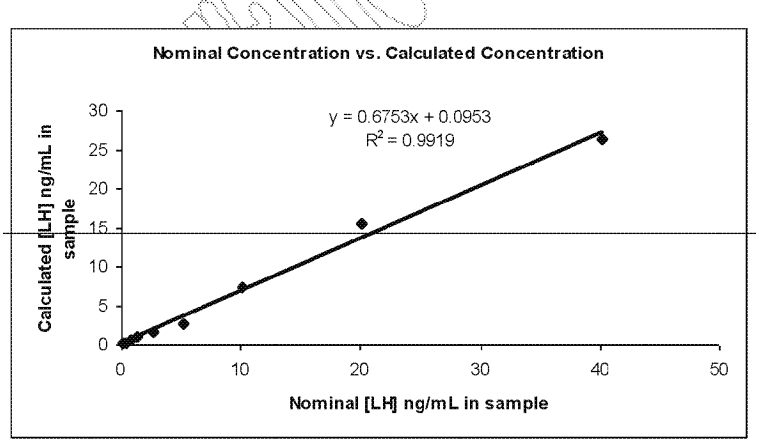
8. Dilution Linearity in Blood

[TC "Dilution Linearity in Blood" \f C \l "1"]

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



Formatted: Font color: Red

Formatted: Left, Indent: Before: 0"

Formatted: Indent: Before: 0"

theranos®
redefining healthcare

9.8. Whole Blood Spike Recovery[TC "Whole Blood Spike Recovery" \f C \l "1"]

Whole blood spike recovery experiment was conducted in samples from three subjects to see if there was variability in recovery. Average percentage recovery was 7177%. Plasma recovered from spiked whole blood, however, gave a higher recovery as compared to the nominal in whole blood. Plasma gave recovery at roughly 134130% 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.

Theranos Internal Only

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

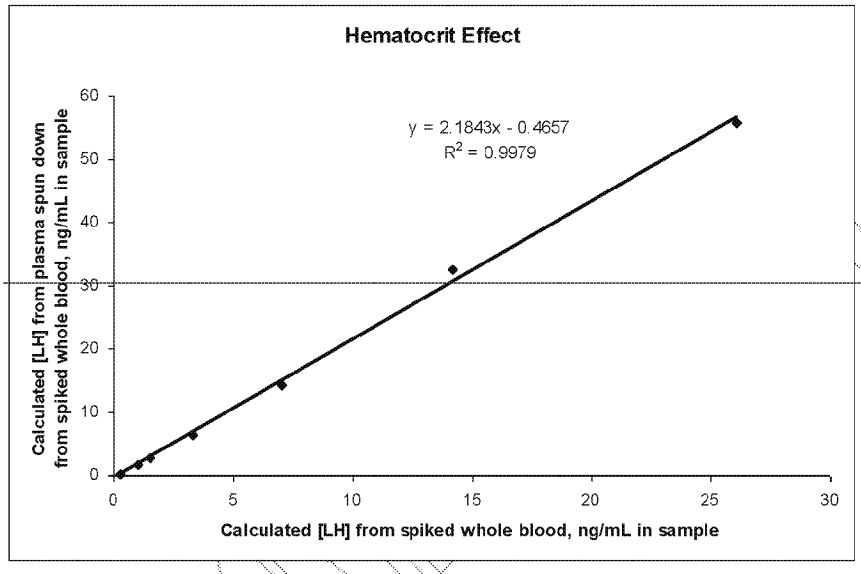
Whole Blood Spike Recovery

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	40.47	820415			39.94	99
2	20.47	427943	476	0	15.73	77
3	10.47	255553	31228	12	8.16	78
4	5.47	138956	21880	16	4.11	75
5	2.47	55652	732	1	1.74	70
6	1.47	22745	8399	37	0.93	63
7	0.97	13264	2295	17	0.70	72
8	0.57	4659	498	11	0.51	89
9	0.47	2497	410	16	0.46	97

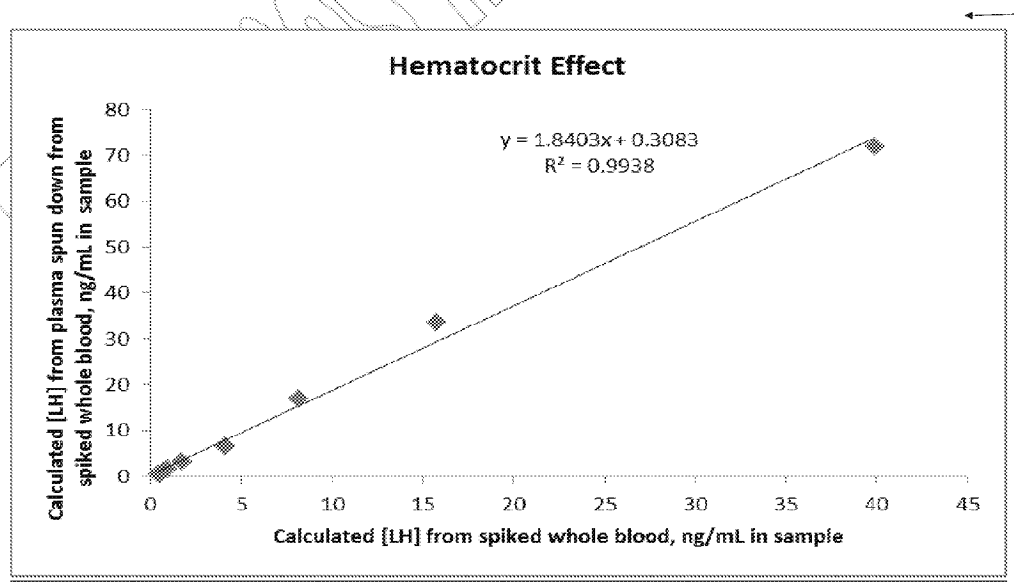
Plasma from Spiked Whole Blood

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	40.47	1193421	62439	5	71.95	178
2	20.47	732295	18917	3	33.65	164
3	10.47	450429	10032	2	16.85	161
4	5.47	209302	7453	4	6.45	118
5	2.47	109170	2567	2	3.21	130
6	1.47	48423	5968	12	1.56	106
7	0.97	27760	1323	5	1.04	108
8	0.57	6856	1504	22	0.56	97
9	0.47	3807	872	23	0.49	104

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



Formatted: Justified, Indent: Before: 0.25"



[SHAPE * MERGEFORMAT]

b. Sample # 2

theranos®
redefining healthcare

Whole Blood Spike Recovery

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.16	422804	33291	8	15.77	78
2	10.16	245408	44100	18	8.78	86
3	2.16	54902	9428	17	1.79	83
4	0.66	16870	1549	9	0.46	71
5	0.26	6218	856	14	0.09	37
6	0.16	3611	330	9	0.00	NA

Plasma from Spiked blood

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.16	782958	42445	5	31.38	156
2	10.16	375727	23299	6	13.87	137
3	2.16	106998	10227	10	3.65	169
4	0.66	31734	4305	14	0.98	150
5	0.26	12356	1098	9	0.31	120
6	0.16	5652	1029	18	0.07	NA

Whole Blood Spike Recovery

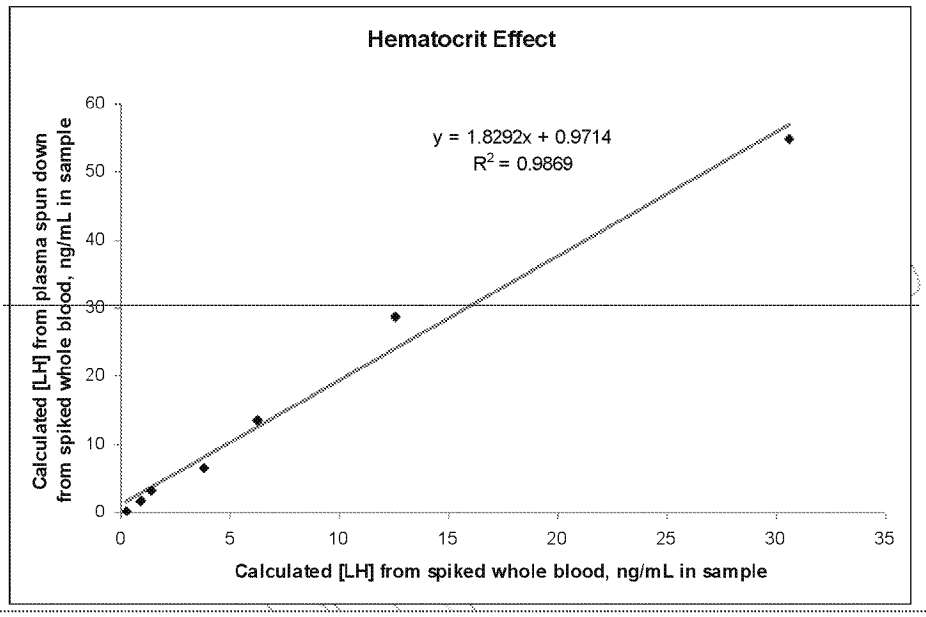
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.24	456998	88440	19	30.59	76
2	20.24	193586	16596	9	12.57	62
3	10.24	97001	5668	6	6.28	61
4	5.24	57953	6348	11	3.79	72
5	2.24	19773	4481	23	1.39	62
6	1.24	12493	1453	12	0.93	75
7	0.24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

Plasma from Spiked Whole Blood

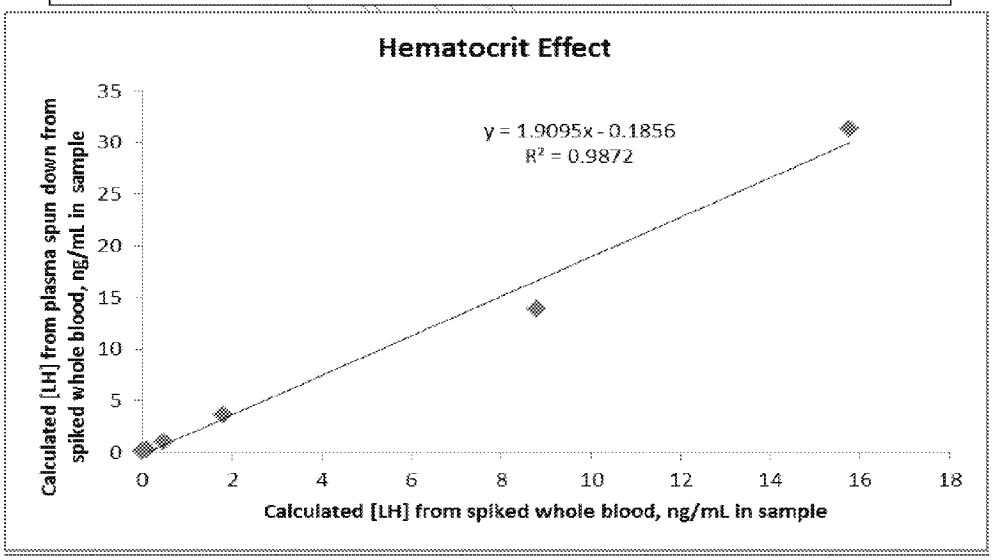
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.3	787033	33051	4	54.99	136
2	20.3	429549	25799	6	28.65	141
3	10.3	209075	12491	6	13.59	132
4	5.3	101721	5735	6	6.59	124
5	2.3	47481	3612	8	3.13	136
6	1.3	23103	1588	7	1.6	122
7	0.3	2455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

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

Theranos Confidential



Formatted: Left, Indent: First line: 0.25"



c. Sample # 3

theranos®
redefining healthcare

Whole Blood Spike Recovery

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.34	518156	38845	7	19.72	97
2	10.34	236521	17255	7	8.44	82
3	2.34	62626	5535	9	2.07	88
4	0.84	19548	1500	8	0.56	66
5	0.44	11250	839	7	0.27	61
6	0.34	6940	507	7	0.12	NA
						79

Plasma from Spiked blood

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.34	695425	28865	4	27.41	135
2	10.34	401272	61663	15	14.90	144
3	2.34	125961	12648	10	4.34	186
4	0.84	36215	7282	20	1.14	136
5	0.44	17537	2220	13	0.49	111
6	0.34	11360	2307	20	0.27	NA

Whole Blood Spike Recovery

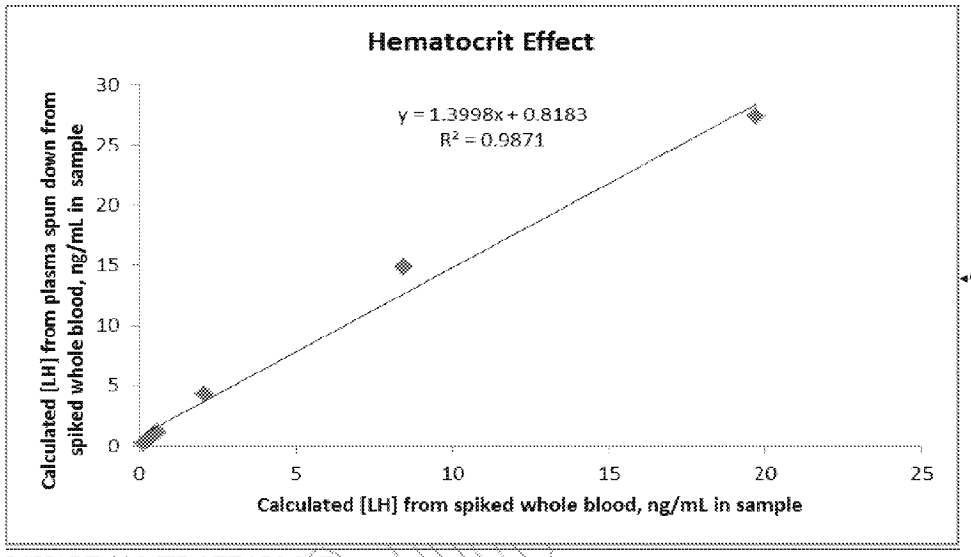
Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std.Dev.	CV%	Calculated [LH] ng/mL in sample	Percentage Recovery (%)
1	40.26	490119	29904	6	32.94	82
2	20.26	217443	21225	10	14.15	70
3	10.26	104291	14073	13	6.75	66
4	5.26	59499	4329	7	3.89	74
5	2.26	23354	1103	5	1.61	71
6	1.26	11222	702	6	0.85	68
7	0.26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

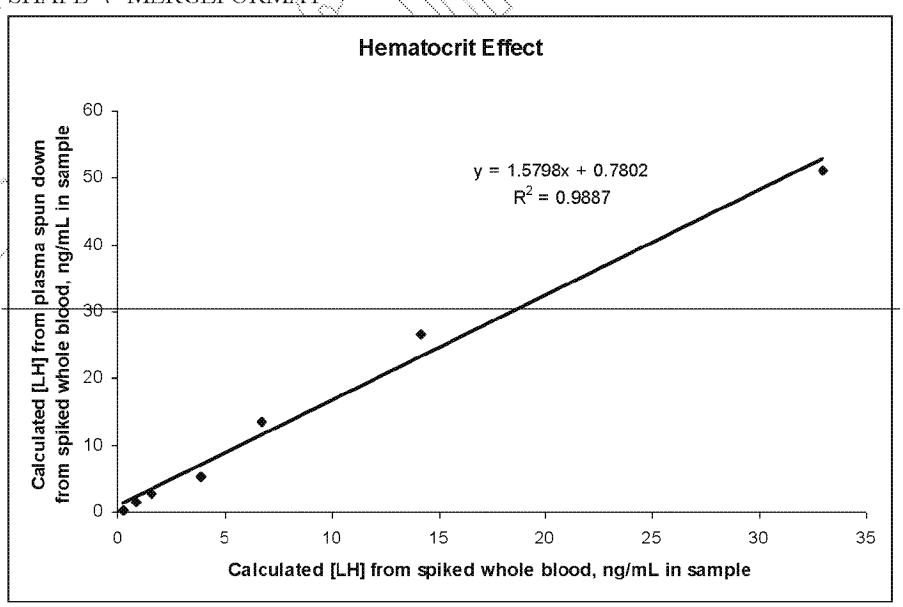
Note: Nominal concentration is spiked concentration plus calculated endogenous level.

Theranos Confidential



Formatted: Left

[SHAPE * MERGEFORMAT



]

~~10.9.~~ **Plasma Spike Recovery**

[TC "Plasma Spike Recovery" \f C \l "1"]

Plasma samples with low endogenous LH levels were used for the spike recovery test. The spike recovery was tested for ~~four~~ three 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~~76%.

a. Sample # 1

	Nominal [LH]				Calculated [LH] ng/mL	% Recovery
Sample	ng/mL in sample	Mean RLU	Std. Dev.	CV%	in sample	
1	40.50	832868	29518	4	40.86	101
2	20.50	432442	33748	8	15.95	78
3	10.50	222945	222945	21	6.94	66
4	5.50	117495	117495	16	3.46	63
5	2.50	46563	46563	21	1.51	60
6	1.50	27007	27007	14	1.03	68
7	1.00	11797	11797	26	0.67	67
8	0.60	5139	5139	13	0.52	86
9	0.50	3710	3710	19	0.48	97

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**~~

b.

Formatted: Numbered + Level: 2 + Numbering Style: a, b, c, … + Start at: 1 + Alignment: Left + Aligned at: 0.75" + Tab after: 1" + Indent at: 1"

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.25	467250	68154	15	17.59	87
2	10.25	252822	37841	15	9.07	88
3	2.25	63619	5980	9	2.10	93
4	0.75	22407	3071	14	0.66	87
5	0.35	10306	1122	11	0.24	67
6	0.25	5978	828	14	0.09	NA

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

Formatted: Font: Bold

Sample	Nominal [LH] ng/mL in sample	Mean RLU	Std. Dev.	CV%	Calculated [LH] ng/mL in sample	% Recovery
1	20.46	514622	64070	12	19.57	96
2	10.46	242464	33610	14	8.67	83
3	2.46	63981	6052	9	2.11	86
4	0.96	23307	492	2	0.69	72
5	0.56	14342	1990	14	0.38	67
6	0.46	13022	2405	18	0.33	NA

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**~~

Theranos Confidential

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.**~~

Formatted: Indent: Before: 0"

Formatted: Indent: Before: 0"

Formatted: Tab stops: Not at 0.75"

~~11.~~ [XE "Matrix Effects"]~~Matrix Effects~~

Formatted: Indent: Before: -0.75"

[TC " Matrix Effects" \f C \l "1"]

~~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.~~

Formatted: Indent: Before: 0"

~~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~~

Theranos Internal Only

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

Theranos Confidential

~~12. Determination of LLOQ and ULOQ~~[TC " Determination of LLOQ and ULOQ" \f C \l "1"]

Formatted: Indent: Before: -0.75"

~~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~~

Formatted: None, Indent: Before: 0.26"

Formatted: Indent: Before: 0"

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

Formatted: None, Indent: Before: 0.26"

Theranos Internal Only

~~13.10.~~ Validation in Clinical Samples[TC "Validation in Clinical Samples" \f C \l "1"]

A total of ~~thirty-five~~fifty-seven 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.

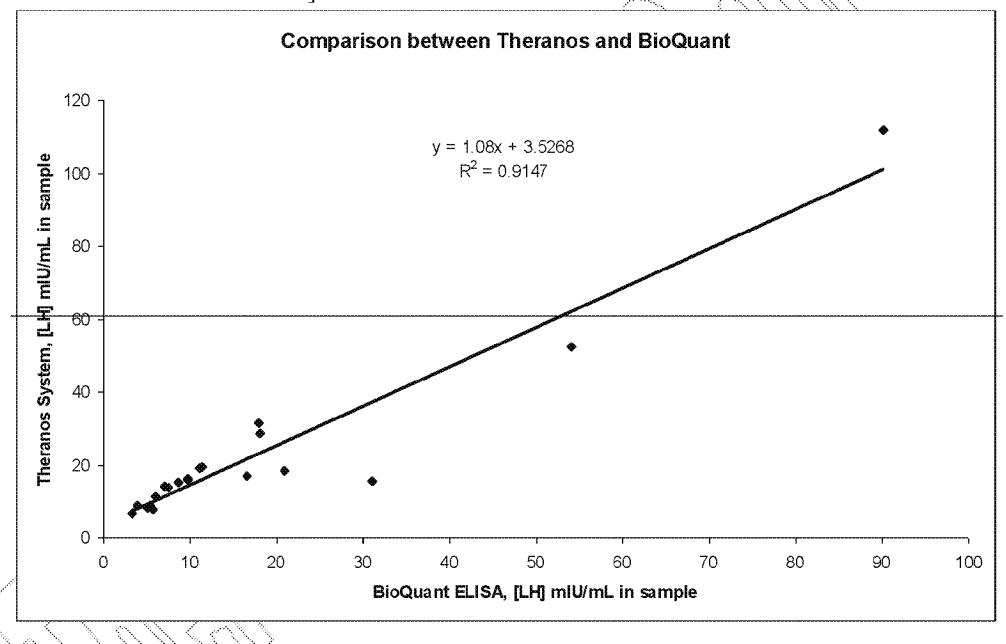
Note: The Theranos LII assay is specific for LII and does not show any cross reactivity with hCG(which is similar in structure). However the commercial LH ELISA kits tested showed high levels of cross reactivity (data not shown) with hCG. This explains why the pregnancy samples are detected as OORL in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

Theranos Internal Only

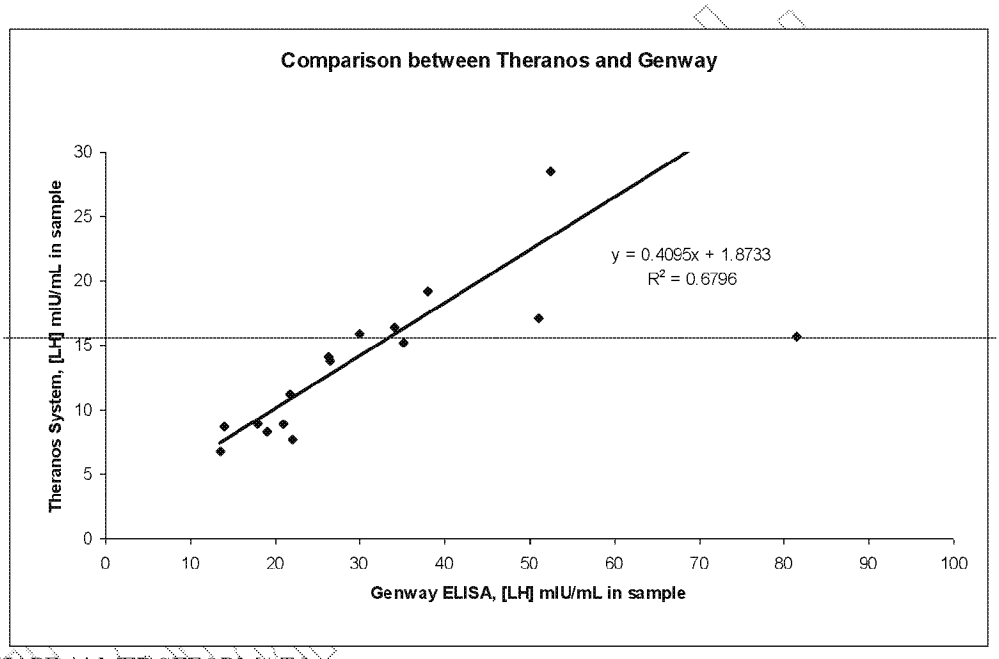
The following graph shows the correlation between Bioquant ELISA kit versus Theranos system for menstrual and post menstrual samples.

[SHAPE * MERGEFORMAT]

Formatted: Indent: Before: 0"



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



[SHAPE * MERGEFORMAT]

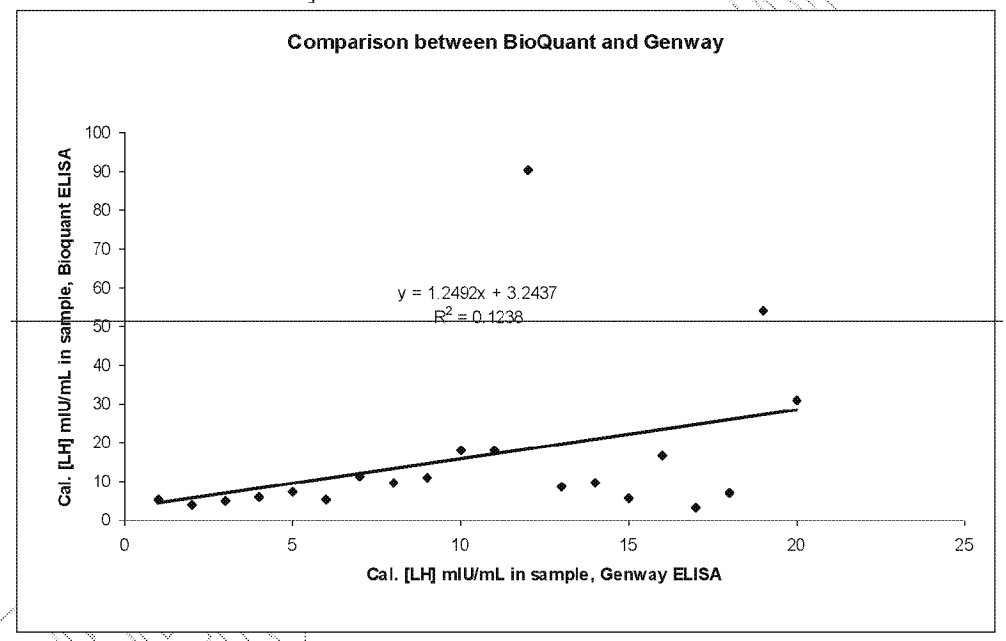
Formatted: Indent: Before: 0"

Formatted: Indent: Before: 0"

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

Formatted: Indent: Before: 0"

[SHAPE * MERGEFORMAT]



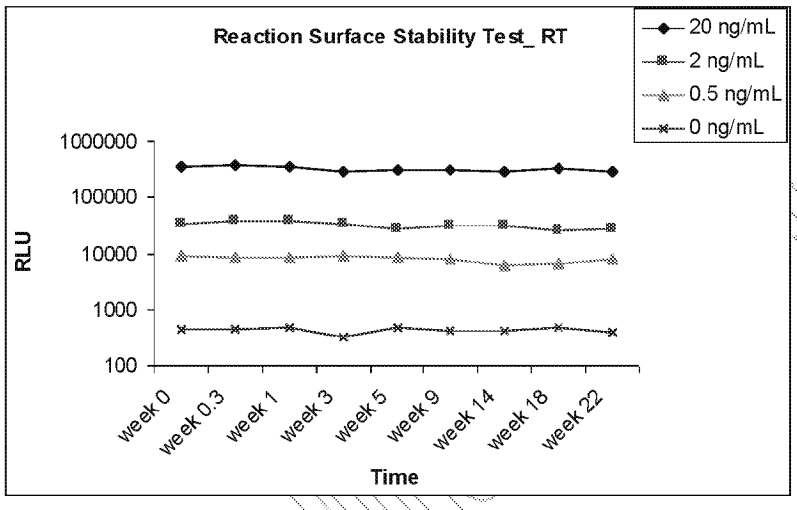
14.11. Stability of key reagents[TC " Stability of key Reagents" \f C \l "1"]

a. Reaction Surface Stability

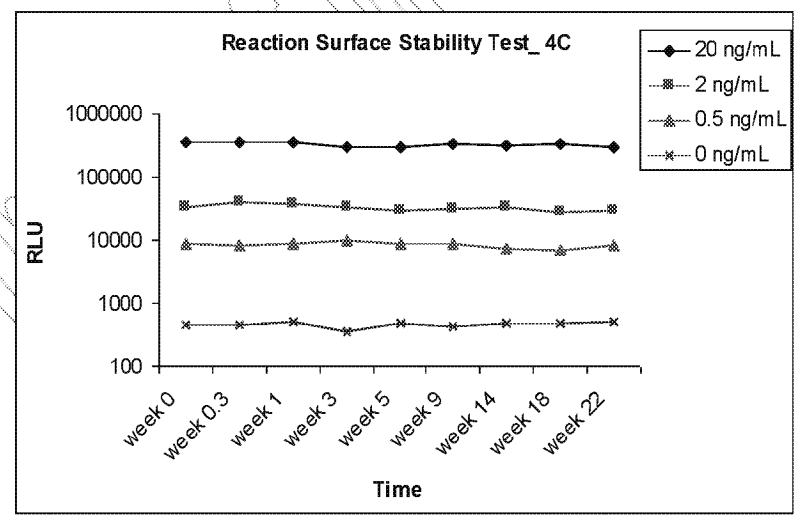
A 24 weeks stability test was set up for the reaction surface and evaluated on Theranos system. The reaction surface stability was tested on four different LH levels: 20, 2, 0.5, and 0 ng/mL. The stability of the reaction surface is tested at two temperature conditions, 4°C and room temperature. Analyte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

Formatted: Indent: Before: 0"

Theranos Internal Only



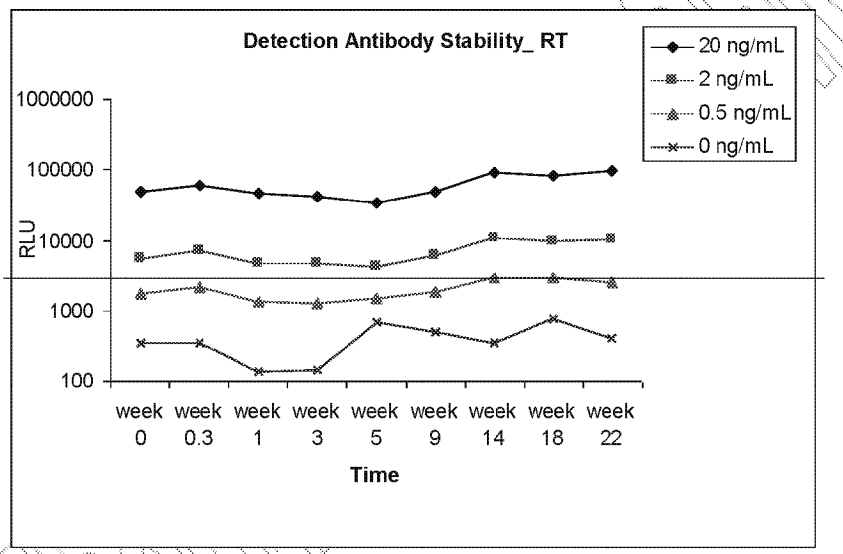
Formatted: Centered

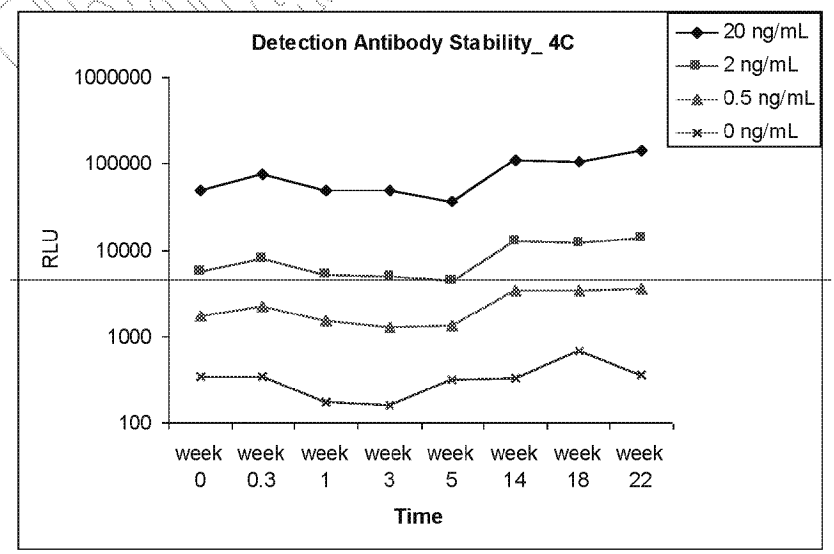


Formatted: Centered

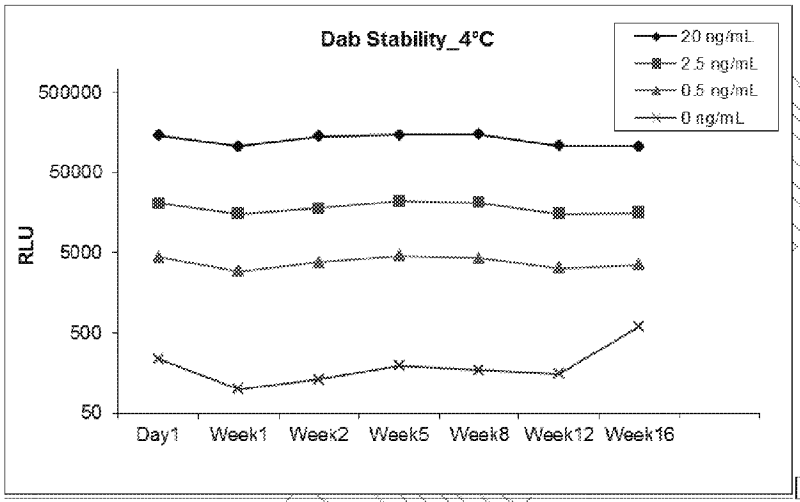
b. Detection Antibody Stability

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 16~~22~~ weeks are shown below.

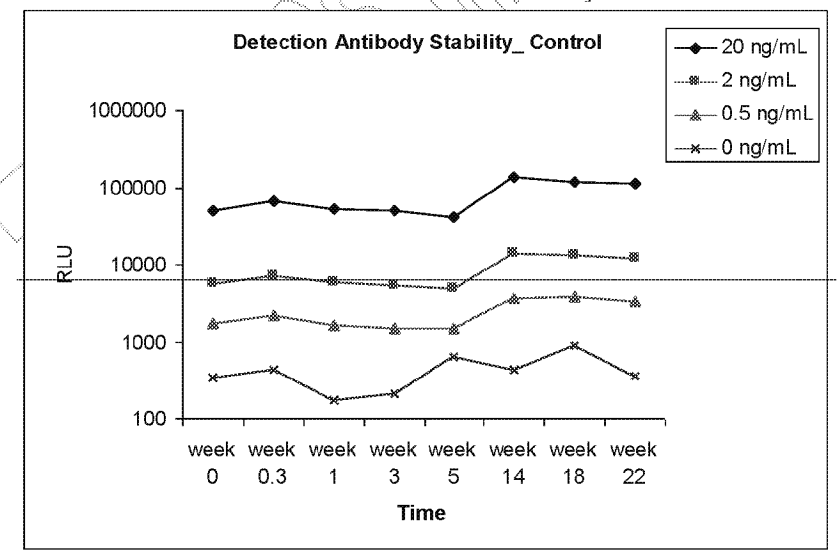


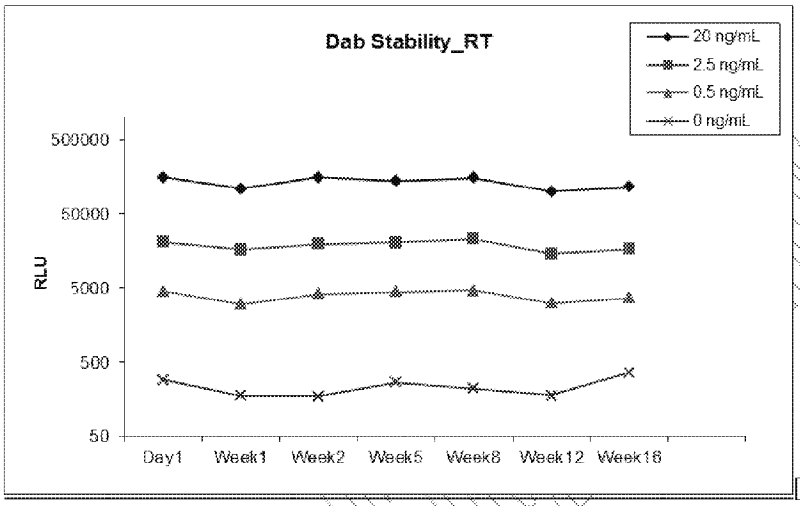


Formatted: Font: Bold, Font color: Red



[SHAPE * MERGEFORMAT]





[SHAPE * MERGEFORMAT]

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

Theranos Confidential

REFERENCE[TC "Reference" \f C \l "1"]

1. R. Penny, H. Guyda, A. Bahdassarian, A. Johanson,and R. Blizzard. Correlation of Serum Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) as Measured by Radioimmunoassay in Disorders of Sexual Development. *The Journal of Clinical Investigation* (1970). **49**:1847

2. P. Chanson, J. Pantel, J. Young, B. Couzinet, J.M. Bidart, and G. Schaison. Free Luteinizing-Hormone Beta-Subunit in Normal Subjects and Patients with Pituitary Adenomas. *Journal of Clinical Endocrinology and Metabolism* (1997). **82**: 1397-1402

3. R A Gadkari, S Roy, N Rekha1, N Srinivasan1 and R R Dighe. Identification of a heterodimer-specific epitope present in human chorionic gonadotrophin (hCG) using a monoclonal antibody that can distinguish between hCG and human LH. *Journal of Molecular Endocrinology* (2005). **34**, 879–887

4. Immuno-Biological Laboratories, Inc. (IBL-America). Luteinizing ELISA kit info.

5. A. Olivares, M. Cardenas, C. Timossi, T. Zarinan, V. Diaz-Sanchez, and U. Ulloa-Aguirre. Reactivity of different LH and FSH standards and preparations in the World Health Organization matched reagents for enzyme-linked immunoassays of gonadotrophins. *Human Reproduction* (2000). **15**: 2285-2291

6. A. Hartree and A. Renwiekt. Molecular structures of glycoprotein hormones and functions of their carbohydrate components. *Biochem. J.* (1992) 287, 665-679

THERANOS CONFIDENTIAL