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Human Chorionic Gonadotropin
Assay Development Report
May 22, 2010

TABLE OF CONTENTS

A. [HYPERLINK \l "_Toc264008089"]
- [HYPERLINK \l "_Toc264008090"]
- [HYPERLINK \l "_Toc264008091"]
- [HYPERLINK \l "_Toc264008092"]
- [HYPERLINK \l "_Toc264008093"]
- [HYPERLINK \l "_Toc264008094"]
- [HYPERLINK \l "_Toc264008095"]
- [HYPERLINK \l "_Toc264008096"]
- [HYPERLINK \l "_Toc264008097"]
- [HYPERLINK \l "_Toc264008098"]
- [HYPERLINK \l "_Toc264008099"]

B. Assay Development and Validation
- Antibody screen
- Capture Antibody Titration
- Detection Antibody Titration
- Interference Test
- Cross Reactivity Test
- Control comparison
- Precision test across three different reagent lots
- Precision test across different instruments
- Dilution linearity
- Whole Blood Screen
- Plasma Screen
- Whole blood and plasma spike recovery
- Matrix Effect
- Hematocrit effect
- Extended Range
- Selectivity Test
- Clinical samples
- Stability
- Urine matrix effect
- Effect of assay time

I. Assay Information

1. Analyte background

Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally produced by the placenta during pregnancy. The hCG molecule consists of two combined, dissimilar subunits designated alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 daltons, confers biological and immunological specificity to the entire hCG molecule by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH).

2. Assay specifications

The Theranos assay for hCG is a sandwich ELISA, specific to pure native human hCG protein.
The ULOQ of the assay is 2000mIU/ml and LLOQ is 2mIU/ml

Reportable ranges are:

Sample type	Low, mIU/ml	High, mIU/ml
Human plasma	3.9	2000

3. Reference assays

1. DSL inc. cat# DSL-10-8300

This is an Enzymatically amplified "two step" sandwich-type immunoassay on microtiter plates. The assay range is 10000 – 5 mIU/ml. The assay time is approximately 1 hour. This kit has been used in-house to validate the Theranos assay.

4. Antibody screening for matched pair

Antibody screening was carried out in 384 well micro-titer plates, 10/10/10 assay time at room temperature and read on the M5.

Table 1: Information of screened antibodies

Number	Company	Epitope	Clonality	Cat #	lot #	Clone
#1	Abcam	NA	Pab (goat)	ab33605	669717	
#2	Abcam	hCG-beta	Mab	ab11388	665320	INN-hCG-2
#3	Abcam	hCG-beta	Mab	ab11390	665323	INN-hCG-24
#4	Abcam	free HCG-beta	Mab	ab11391	665324	INN-hCG-68
#5	Abcam	hCG b cf	Mab	ab11382	665318	INN-hCG-106
#6	Abcam	hCG-beta	Mab	ab9228		BCI151
#7	GenWay	NA	Mab	20-511-240950	10126	218-10148
#8	GenWay	NA	Pab (goat)	18-511-244910	8J29706	
#9	Abcam	hCG-beta	Mab	ab9229	691847	BCI152
#10	Abcam	hCG-beta	Mab	ab7907	682161	HCG-55
#11	Abcam	hCG-beta	Mab	ab764		HCG-61

	Dab 1	Dab 2	Dab 3	Dab 5	Dab 6	Dab 7	Dab 8	Dab 9	Dab 10	Dab 11
Cab 1										
Cab 2										
Cab 3										
Cab 5										
Cab 6										
Cab 7										
Cab 8										
Cab 9										
Cab 10										
Cab 11										

Number of Capture antibody tested: 10
Number of Detection antibody tested: 11
Total Number of antibody pairs tested:
Good dose response
OK dose responses
Poor modulation or no modulation

Figure 1: Chosen pair Ab graph

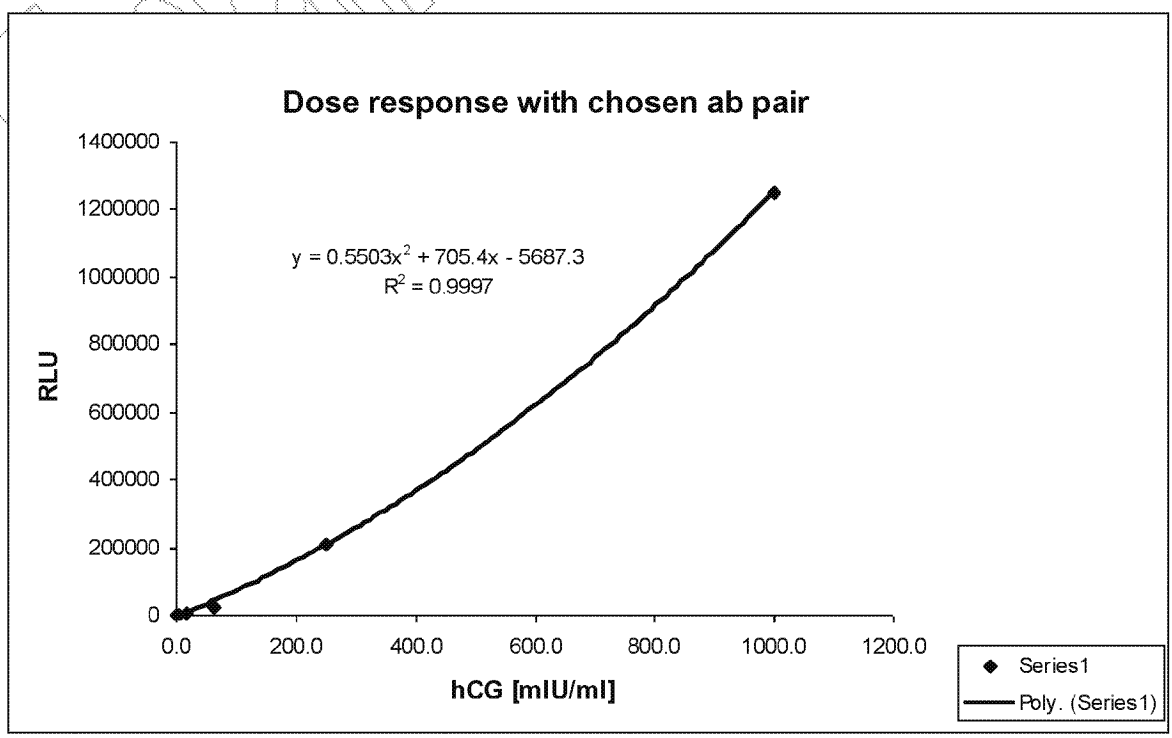


Table 2: Chosen pair Ab data

Conc.in sample (mIU/ml)	MeanValue	Std.Dev.	CV%
1000.0	1249752	25810	2
250.0	210343	5861	3
62.5	23400	599	3
15.6	5059	125	2
3.9	2164	38	2
0.0	1741	153	4
S/B	718		
Ave. CV%	3		

5. Assay Reagents

A. Capture Antibody

Vendor	abcam
Catalog #	ab764
Type	Mouse monoclonal to hCG beta
Specificity	React with beta subunit of hCG. Cross-reactivity: hLH < 0.86%; FSH > 0.5%; hTSH >0.5
Stock Conc.	In liquid form at concentration of 1mg/ml in PBS pH 7.4 containing 15mM Sodium Azide Biotin conjugated: 0.5 mg/mL
Storage	Aliquot and store at -20 C or -80. If aliquoted for long term storage, avoid repeated freeze/thaw cycles.
Data Sheet	

B. Detection Antibody

Vendor	abcam
Catalog #	9229
Type	Mouse monoclonal to hCG beta
Specificity	Specific for hCG beta
Stock Conc.	In liquid form at concentration of 1mg/ml in PBS pH 7.4 containing 15mM Sodium Azide. AP conjugated: 0.5mg/ml
Storage	Aliquot and store at -20 C or -80. If aliquoted for long term storage, Avoid repeated freeze/thaw cycles.
Data Sheet	

C. Analyte

Vendor	abazyme
Catalog #	HOR-250
Current lot #	107HCG01
Mol Wt	
Stock Conc.	In Lyophilized form. Reconstituted in PBS pH 7.4 to the concentration of 10mg/ml then diluted to 1mg/ml in PBS w/0.1 %BSA
Storage	Store lyophilized product at -20 C. After reconstitution, aliquot and store at -20 C. Avoid multiple freeze/thaw cycles.
Data sheet	

6. Reagent Handling and Storage

Please see handling and storage conditions of capture, detection Ab, and analyte above. Biotin and Alkaline Phosphatase conjugates were stored at 4°C in Dojindo storage buffers. Since Dojindo specifies that the conjugates are stable for at least two months at 0-5°C; the conjugates were qualified at the end of three months either against a new conjugate or compared to historical data.

7. Protocols

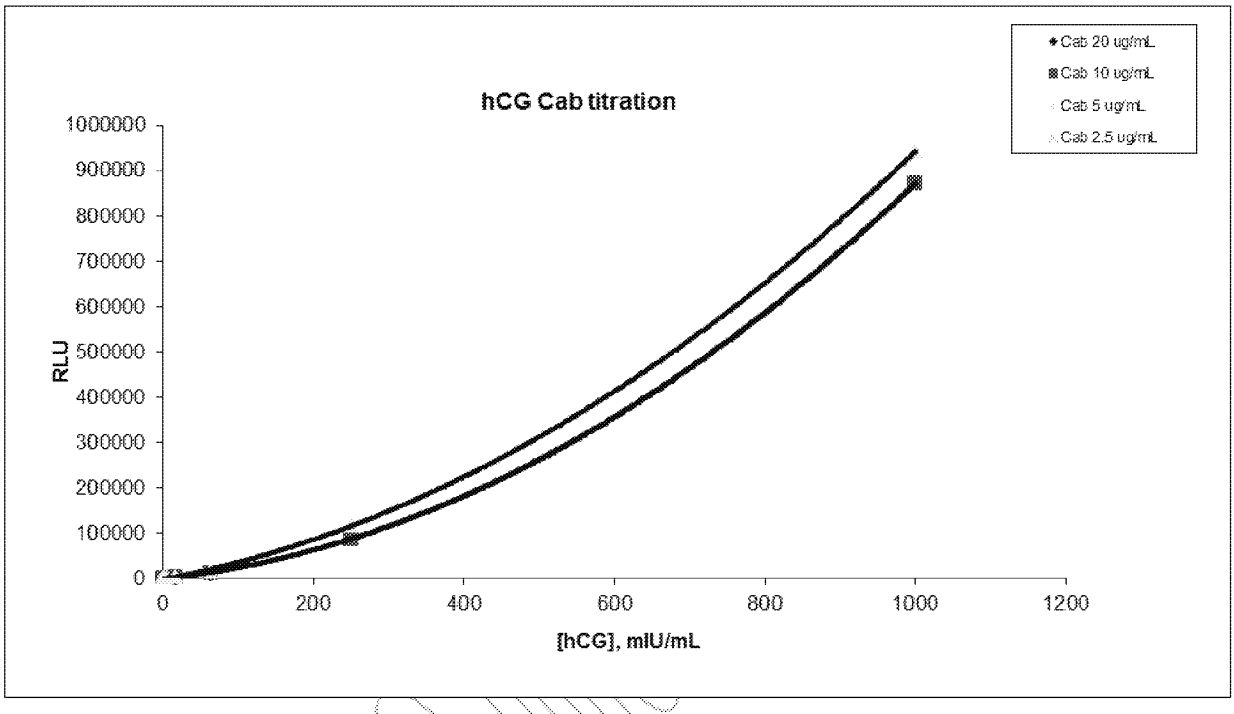
Protocol name	Svn #	Sample dilutions	Tip positions	Post sample wash	Post sample wash
Generic_10_PSW	2089	10X	Tips 1-6	2 times	2 times

II. Assay Optimization

1. Capture Antibody Titration

Capture antibody was titrated at 2.5, 5, 10, 20ug/mL on Theranos Systems. A concentration of 5ug/mL is the final pick of Cab concentration for Theranos assay.

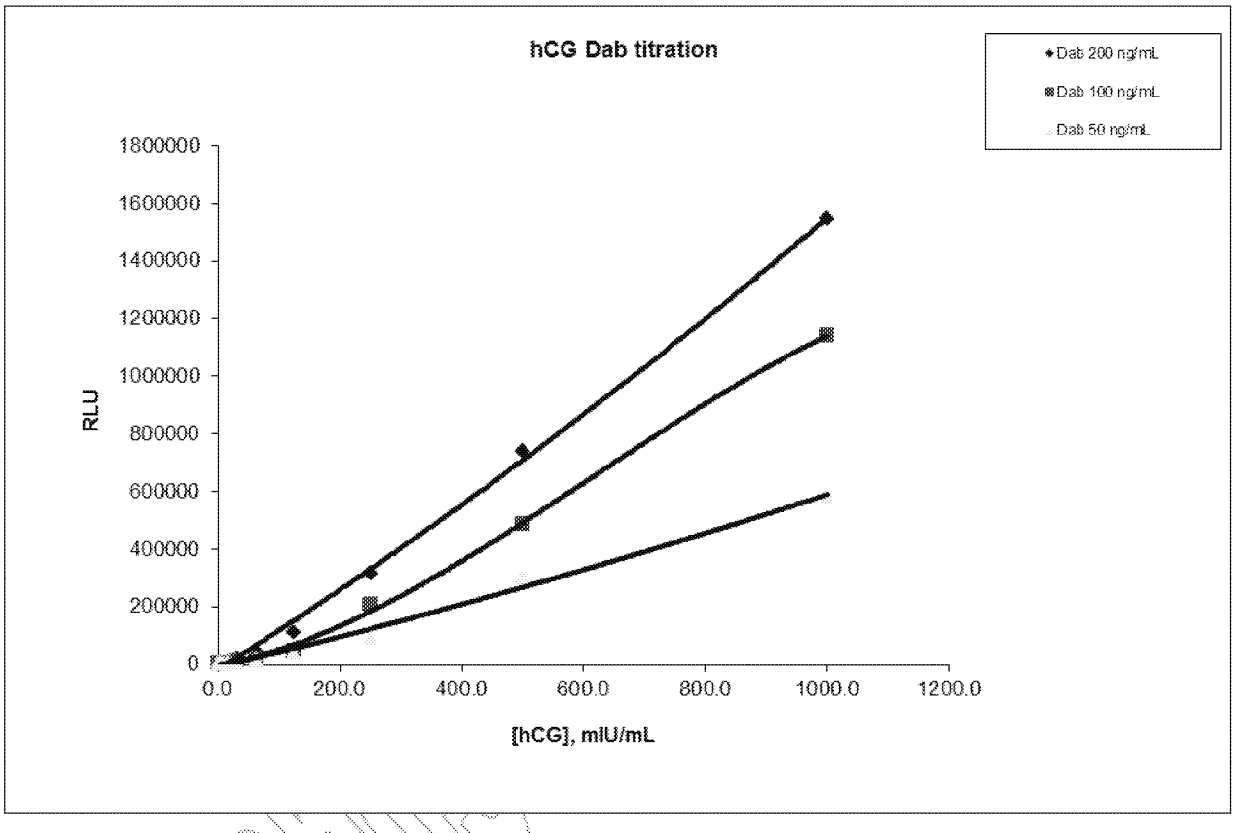
Cab	hCG mIU/ml	Mean RLU	StDev	%CV	S/B
20ug/ml	1000	872460	82677	9	920
	250	89980	5575	6	
	62.5	10354	843	8	
	15.6	2640	499	19	
	3.9	1247	121	10	
	1.95	1064	139	13	
	0	948	90	9	
10ug/ml	1000	871168	29325	3	891
	250	88426	21217	24	
	62.5	11760	1683	14	
	15.6	3013	388	13	
	3.9	1358	99	7	
	1.95	1151	70	6	
	0	978	159	16	
5ug/ml	1000	942109	57712	6	846
	250	118924	8331	7	
	62.5	13888	1258	9	
	15.6	3428	633	18	
	3.9	1648	87	5	
	1.95	1316	76	6	
	0	1113	86	8	
2.5ug/ml	1000	730151	22042	3	718
	250	116500	19830	17	
	62.5	13555	948	7	
	15.6	3200	422	13	
	3.9	1727	239	14	
	1.95	1255	49	4	
	0	1017	95	9	



2. Detection Antibody Titration

Detection antibody was titrated at 200, 100, 50, 25 ng/mL in Stabilzyme AP Stabilizer on Theranos Systems. A concentration of 100ng/mL is the final pick of Dab concentration for Theranos assay.

Dab	hCG mIU/ml	Mean RLU	StDev	%CV	S/B
200ng/ml	1000	1544872	87736	6	944
	500	740959	98073	13	
	250	317711	11905	4	
	125	112470	6726	6	
	63	44376	6184	14	
	31	18653	1268	7	
	16	8587	868	10	
	8	5879	551	9	
	4	2779	145	5	
	0	1636	490	30	
100ng/ml	1000	1140987	118993	10	1254
	500	485787	34118	7	
	250	205213	35817	17	
	125	46873	6599	14	
	63	21241	3269	15	
	31	9300	1333	14	
	16	4018	545	14	
	8	2149	433	20	
	4	1380	196	14	
	0	910	47	5	
50ng/ml	1000	583665	135346	23	1103
	500	301095	10941	4	
	250	90957	12185	13	
	125	36402	1273	3	
	63	13654	1713	13	
	31	4576	463	10	
	16	2279	468	21	
	8	1399	382	27	
	4	942	69	7	
	0	529	65	12	



3. Interference Test

Tested 4 antibody pairs with the best dose response for Interference with Estradiol, hLH, FSH, Progesterone analytes

Each antibody pair is tested with a 4 point standard curve of the assay specific analyte and with a 4-point standard curve of analyte spiked with excess of the highest level of the interfering substance

Conclusion: with the final chosen antibody pair, tested analytes has no interference with hCG assay.

Table: Interference data with final pick pair Ab

Control : only hCG	Mean	Recovered hCG	
hCG mIU/mL	RLU	mIU/mL	%Recovery
1000.0	2091514	1022	102
62.5	417134	65	104
3.9	21304	3	70
0.0	1230		

hCG + spiked LH	Mean	Recovered hCG	
hCG mIU/mL	RLU	mIU/mL	%Recovery
1000.0	2172310	1097	110
62.5	497381	85	137
3.9	25337	3	78
0.0	3245		

hCG + spiked FSH	Mean	Recovered hCG	
hCG mIU/mL	RLU	mIU/mL	%Recovery
1000.0	2179863	1104	110
62.5	534743	96	153
3.9	29130	3	86
0.0	1762		

hCG + spiked Extrodiol	Mean	Recovered hCG	
hCG mIU/mL	RLU	mIU/mL	%Recovery
1000.0	2140078	1067	107
62.5	460175	76	121
3.9	23614	3	74
0.0	1502		

hCG + spiked Progesterone	Mean	Recovered hCG	
hCG mIU/mL	RLU	mIU/mL	%Recovery
1000.0	2063158	997	100
62.5	481921	81	130
3.9	25143	3	77
0.0	1515		

4. Cross Reactivity

Cross reactivity with related analyte: Test 4 antibody pairs with the best dose response for cross reactivity with Estradiol, hLH, FSH, Progesterone analytes (6 point standard curve of the assay specific analyte and 6 point standard curve of tested analyte

Table: Cross reactivity data with final pick pair Ab

Control hCG [mIU/mL]	RLU	Mean RLU	StDev	%CV
1000	1710481	1720848	14661	0.9
	1731215			
200	995233	994055	1666	0.2
	992877			
40	315940	314944	1408	0.4
	313948			
10	75182	75355	244	0.3
	75527			
3	18028	17579	635	3.6
	17129			
0	1890	1897	10	0.5
	1904			

LH (ng/ml)	RLU	Mean RLU	StDev	%CV
20	717	738	31	4
	760			
10	819	702	165	24
	585			
2	1029	931	138	15
	834			
0.5	868	851	24	3
	834			
0.1	1574	1577	3	0
	1579			
0.0	1404	2379	1379	58
	3354			

Estradiol (ng/mL)	RLU	Mean RLU	StDev	%CV
32	614	604	14	2
	595			
8	785	641	203	32
	497			
2	604	546	83	15
	487			
0.68	1418	1002	589	59
	585			
0.23	712	721	14	2
	731			
0	1019	1150	186	16
	1282			

FSH [mIU/ml]	RLU	Mean RLU	StDev	%CV
200	726	931	290	31
	1136			
40	999	799	283	35
	600			
8	873	773	141	18
	673			
2	629	643	21	3
	658			
0.5	624	729	148	20
	834			
0	663	809	207	26
	955			

Progesterone (ng/mL)	RLU	Mean RLU	StDev	%CV
60	770	1033	372	36
	1297			
15	653	768	162	21
	882			
3.75	863	836	38	5
	809			
0.94	663	924	369	40
	1184			
0.23	741	680	86	13
	619			
0	682	2866	3088	108
	5050			

III. Assay Validation

1. Precision Test

A 10 points of standard curve were assayed on triplicate cartridges (N = 3) of 3 lots to determine precision. 6 points of calibrators were assayed on 18 instruments in replicate on each instrument.

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hCG		RLU								Signal
		Lot1		Lot2		Lot3				
mIU/mL	Cartridge	Tip 1	Tip 2	Tip 1	Tip 2	Tip 1	Tip 2	Mean	Stdev	%CV
1000.0	1	1385410	1391790	1280372	1190322	1299446	1265539	1180423	118234	10
	2	1189986	1273790	1050364	1038618	1095115	1108599			
	3	1190883	1189336	1083521	1056886	1167399	990232			
500.0	1	539630	584584	526844	476044	531217	480152	518489	72101	14
	2	476005	499504	464519	459486	408212	382695			
	3	633068	629010	618957	591440	507900	523531			
250.0	1	168567	179229	197570	172357	171783	248756	181385	24692	14
	2	185612	191456	206241	202375	148511	157603			
	3	169380	193543	197518	167145	160804	146474			
125.0	1	71843	65380	70645	60820	52532	46774	63399	9956	16
	2	64757	63712	56399	59392	55857	57561			
	3	72826	78790	77994	79011	56422	50470			
62.5	1	22347	20741	19880	20120	15222	16606	21859	3629	17
	2	28675	25997	25059	23423	20872	18824			
	3	27654	24420	22623	22859	19186	18948			
31.3	1	7333	7927	8572	7964	6249	6991	8194	1196	15
	2	9880	8797	9297	8016	7032	6207			
	3	8428	9591	10036	9494	8529	7141			
15.6	1	4406	4380	4415	4524	3263	3523	4494	710	16
	2	6103	5536	5280	5040	4877	3680			
	3	4571	4253	4727	4294	4157	3855			
7.8	1	2199	2405	2310	2568	2203	2189	2383	226	9
	2	2705	2076	2012	2414					
	3	2701	2404	2629	2708	2345	2262			
3.9	1	1851	1852	1793	1697	1654	1455	1701	181	11
	2	1586	1483	1664	1794	1539	1299			
	3	1931	1945	1762	1844	1880	1585			
0.0	1	1089	1262	1220	1264	1178	1059	1041	137	13
	2	1052	1083	897	938	1027	856			
	3	948	991	1158	1009	862	839			
						Ave. inter-lot Signal %CV (3 lots)				13

hCG		Observed Concentration [mIU/mL]						
		Intra mean					Conc	
mIU/mL	Cartridge	Lot1	Lot2	Lot3	Inter mean	StDev	%CV	% Recovery
1000.0	1	OORL	1091.7	1093.5	1013.0	62.3	6	101
	2	1052.0	996.6	947.3				
	3	1017.8	975.0	929.9				
500.0	1	541.9	537.2	500.5	517.4	54.4	11	103
	2	487.4	483.1	418.8				
	3	592.3	587.2	507.9				
250.0	1	241.1	253.9	272.0	248.5	17.2	7	99
	2	254.1	263.0	222.1				
	3	247.7	260.1	222.7				
125.0	1	133.9	133.1	109.1	127.6	12.4	10	102
	2	128.5	123.1	118.7				
	3	142.5	145.6	114.3				
62.5	1	63.0	60.4	50.8	63.9	7.5	12	102
	2	74.0	70.7	59.5				
	3	71.6	66.9	57.8				
31.3	1	28.5	30.5	25.1	30.3	3.7	12	97
	2	33.8	32.9	25.1				
	3	32.8	35.2	29.1				
15.6	1	16.8	16.8	12.4	17.2	2.9	17	110
	2	22.3	20.8	16.2				
	3	16.8	17.2	15.1				
7.8	1	7.2	7.5	6.6	7.3	0.8	12	94
	2	7.6	5.8	OORL				
	3	8.4	8.3	7.2				
3.9	1	4.8	4.6	3.7	4.3	0.7	**15**	**111**
	2	3.4	3.8	OORL				
	3	5.3	4.8	4.2				
0.0	1							
	2							
	3							
				Ave. inter-lot concentration %CV (3 lots)				**11.2**

2. %CV Test

Middle point of calibrator (62.5mIU/mL) were assayed 24 times on 24 instruments and replicate on each instrument to determine the mid-range %CV

hCG calibrator=62.5mIU/ml					Total inter cartridge signal %CV			Total inter cartridge concentration %CV			
								Recovered hCG [mIU/ml]			
Cartridge	RLU	Intra Mean	Stdev	Intra %CV	Inter Mean	Stdev	%CV	Intra Mean	Inter Mean	Stdev	%CV
1	28685	29195	721	2.5	29407	3531	12.0	62.2	62.3	5.0	8.0
	29704										
2	29756	27971	2524	9.0				60.2			
	26186										
3	33243	31486	2484	7.9				65.5			
	29730										
4	31436	31222	302	1.0				65.2			
	31009										
5	36655	38072	2003	5.3				74.8			
	39488										
6	28071	27948	173	0.6				60.2			
	27826										
7	30216	28822	1972	6.8				61.6			
	27428										
8	32119	30712	1990	6.5				64.4			
	29305										
9	27760	29007	1763	6.1				61.8			
	30254										
10	31717	31195	738	2.4				65.2			
	30673										
11	23939	27349	4823	17.6				59.1			
	30759										
12	33793	32615	1666	5.1				67.2			
	31437										
13	28246	28051	276	1.0				60.4			
	27856										
14	32464	32567	146	0.4				67.2			
	32671										
15	27262	27472	297	1.1				59.5			
	27682										
16	31063	30267	1126	3.7				63.8			
	29470										
17	22861	23148	406	1.8				52.4			
	23435										
18	23798	24703	1280	5.2				55.0			
	25608										
19	29439	27902	2173	7.8				60.1			
	26366										
20	28727	26009	3844	14.8				57.0			
	23291										
21`	33911	32224	2387	7.4				66.6			
	30536										
22	32512	31547	1365	4.3				65.7			
	30583										
23	26406	24203	3115	12.9				54.1			
	22001										
24	32871	32091	1102	3.4				66.5			
	31312										

3. Confirming Assay Standards

The analyte used for the Theranos assay was tested in reference ELISA kit. Average recovery was 115% and slope of Nominal versus Calculated was 1.1

Nominal hCG				Recovered hCG	
mIU/ml	Mean OD	StDev	%CV	mIU/ml	%Recovery
1000	1.233	0.047	4	917	92
300	0.797	0.018	2	400	133
100	0.381	0.008	2	104	104
30	0.166	0.008	5	27	89
10	0.075	0.006	7	10	96
3	0.041	0.004	9	6	OORH
0	0.031	0.001	3.2		
				Average %Recovery	103

4. Dilution Linearity

Pregnancy Sample #63 (Measured concentration: 11921mIU/ml) 9X diluted to 1324.6mIU/ml as first test concentration points then was serially 3-fold diluted with normal male serum sample which has no detectable level of hCG to yield sample concentrations within the dynamic range of the assay. The following equation was used to determine the recovery percentage: 100*(calculated concentration/Nominal concentration)

Dilution Linearity Summary:

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
1324.6	1325.9	1423822	30469	2	1189.3	31	3	90
441.5	442.8	378715	51013	13	329.6	34	10	74
147.2	148.5	71510	9420	13	112.1	9	8	75
49.1	50.4	12354	1373	11	33.5	3	10	67
16.4	17.7	3296	331	10	7.9	1	14	45
0.0	1.3	1123	128	11	1.3	0	22	
						Ave. % Recovery		70
						Ave. Conc %CV		11

3. Whole Blood Screen

10 blood samples were screened.

Samples	Mean RLU	StDev	%CV	Cal. hCG mIU/ml
1	1095	47.8	4.4	2.5
2	855	0.0	0.0	1.6
3	644	32.1	5.0	0.9
4	702	55.6	7.9	1.1
5	1000	20.9	2.1	2.1
6	981	61.0	6.2	2.0
7	1009	175.2	17.4	1.0
8	1228	239.3	19.5	1.5
9	1154	39.6	3.4	1.4
10	1236	172.2	13.9	1.6

4. Plasma Screen

10 blood samples were screened.

Plasma was generated by spinning down whole blood obtained from Stanford blood bank at 10,000rpm in for 10mins.

Samples	Mean RLU	StDev	%CV	Cal. hCG mIU/ml
1	866	2.8	0.3	1.6
2	972	39.8	4.1	2.0
3	670	19.0	2.8	1.0
4	832	59.9	7.2	1.5
5	915	109.9	12.0	1.8
6	1006	43.9	4.4	2.1
7	1327	203	15	1.8
8	1168	98	8	1.4
9	1180	117	10	1.4
10	1016	76	7	1.0

5. Spike Recovery (spike into whole blood and plasma)

10X concentrated hCG assay buffer calibrators were directly spiked into whole blood and plasma for a total of 12 standard points over the range of 2-2000 mIU/ml. Tested the recovery of spiked whole blood and spiked plasma (spike into plasma directly). The recovery was estimated with plasma calibration curve.

#1 whole blood sample

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
2000	2005	1510157	177083	12	1621.004	185.133	11	81
1000	1005	787092	99797	13	885.932	98.464	11	88
500	505	305564	43736	14	430.733	53.163	12	85
250	255	109780	21638	20	212.910	25.234	12	84
125	130	35598	6532	18	111.620	12.157	11	86
63	67	12407	1733	14	55.878	6.064	11	83
31.3	36	5807	532	9	27.986	2.789	10	77
15.6	21	3825	420	11	16.989	2.469	15	83
7.8	13	2598	78	3	9.690	0.466	5	76
3.9	9	2144	150	7	7.035	0.862	12	80
2.0	7	2126	420	20	7.244	2.240	31	106
0	5	1607	620		4.917	3.352		
						Ave. % Recovery		84
						Ave. Conc %CV		13

#2 whole blood sample

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
2000	2004	1601697	98427	6	1716.208	103.009	6	86
1000	1004	650615	39405	6	751.672	38.513	5	75
500	504	262631	60428	23	399.368	60.494	15	79
250	254	97006	8858	9	198.390	10.615	5	78
125	129	32970	8858	28	106.028	17.913	17	82
63	67	10874	8858	38	48.832	17.244	35	73
31.3	35	4837	8858	25	22.598	6.680	30	64
15.6	20	2743	8858	16	10.563	2.678	25	54
7.8	12	2720	8858	12	10.430	1.999	19	88
3.9	8	1875	8858	7	5.523	0.694	13	69
2.0	6	1562	8858	3	3.800	0.288	8	63
0	4	1540	8858	7	4.188	0.617	15	
						Ave. % Recovery		74
						Ave. Conc %CV		16

#1 plasma sample

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
2000	2001	1667830	227142	14	1787.044	240.695	13	89
1000	1001	734020	253257	35	834.271	248.100	30	83
500	501	383291	42368	11	499.994	42.139	8	100
250	251	119765	17917	15	224.571	20.092	9	89
125	126	42613	5669	13	124.080	9.539	8	98
63	64	17216	1599	9	71.100	4.567	6	112
31.3	32	6981	852	12	33.811	4.180	12	104
15.6	17	2915	274	9	11.586	1.643	14	69
7.8	9	1810	226	13	5.186	1.239	24	58
3.9	5	1356	53	4	2.871	0.245	9	57
2.0	3	1259	145	12	2.510	0.567	23	81
0	1	919	64	7	1.141	0.207	18	
						Ave. % Recovery		86
						Ave. Conc %CV		15

#2 plasma sample

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
2000	2001	1966285	172168	9	2104.307	185.361	9	105
1000	1001	773807	55367	7	872.613	54.525	6	87
500	501	390240	72439	19	492.244	64.865	13	98
250	251	112830	15269	14	216.757	17.224	8	86
125	126	40992	15135	37	120	26	22	95
63	64	18014	1568	9	73.353	4.316	6	115
31.3	32	5572	1809	32	26.401	10.131	38	82
15.6	17	3055	380	12	12.427	2.276	18	74
7.8	9	2031	123	6	6.392	0.701	11	71
3.9	5	1618	233	14	4.182	1.214	29	83
2.0	3	1200	70	6	2.220	0.272	12	72
0	1	987	94	10	1.375	0.331	24	
						Ave. % Recovery		88
						Ave. Conc %CV		16

6. Matrix Effect

5 point hCG calibrators were spiked into hemolysed plasma, lipemic serum and icteric serum.). The recovery was estimated with Blocking buffer calibration curve.

hemolyzed, lipemic and icteric matrix has very limited impact on spiked hCG recovery

- hCG % recovery with hemolyzed matrix: 83-93%, average 90%.
- hCG % recovery with lipemic matrix: 73-96%, average 86%.

- hCG % recovery with icteric matrix: 80-108%, average 93%.

Spiked into hemolyzed plasma

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
1000.0	1001.3	1239482	60062	5	932.7	47	5	93
250.0	251.3	225589	33285	15	223.5	22	10	89
62.5	63.8	25761	3306	13	56.6	5	10	89
15.6	16.9	5794	1513	26	14.3	4	30	85
3.9	5.2	1970	195	10	3.3	1	16	63
0.0	1.3	1180	149	13	1.3	0	24	
						Ave. % Recovery		84
						Ave. Conc %CV		16

Spiked into lipemic serum

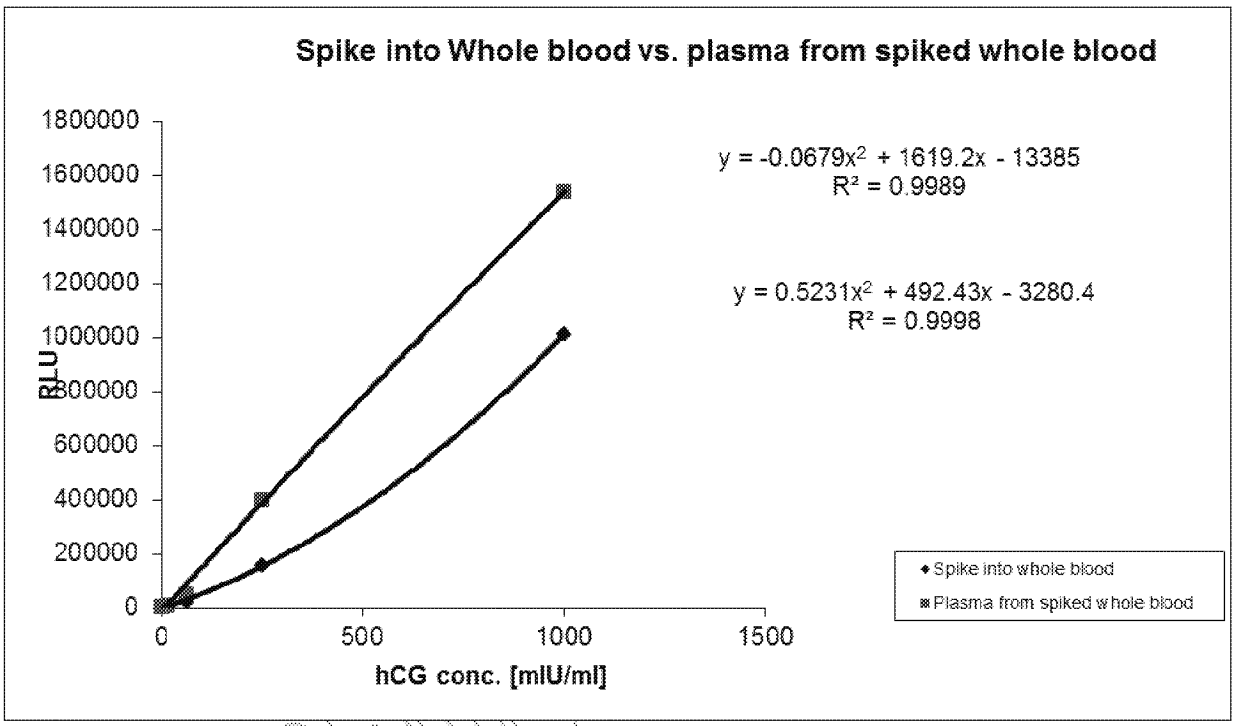
hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
1000.0	1002.1	1259488	100440	8	949.0	80	8	95
250.0	252.1	192688	16388	9	208.6	12	6	83
62.5	64.6	22772	3603	16	51.5	6	12	80
15.6	17.7	4787	393	8	11.5	1	10	65
3.9	6.0	2157	241	11	3.8	1	18	63
0.0	2.1	1532	136	9	2.1	0	15	
						Ave. % Recovery		77
						Ave. Conc %CV		12

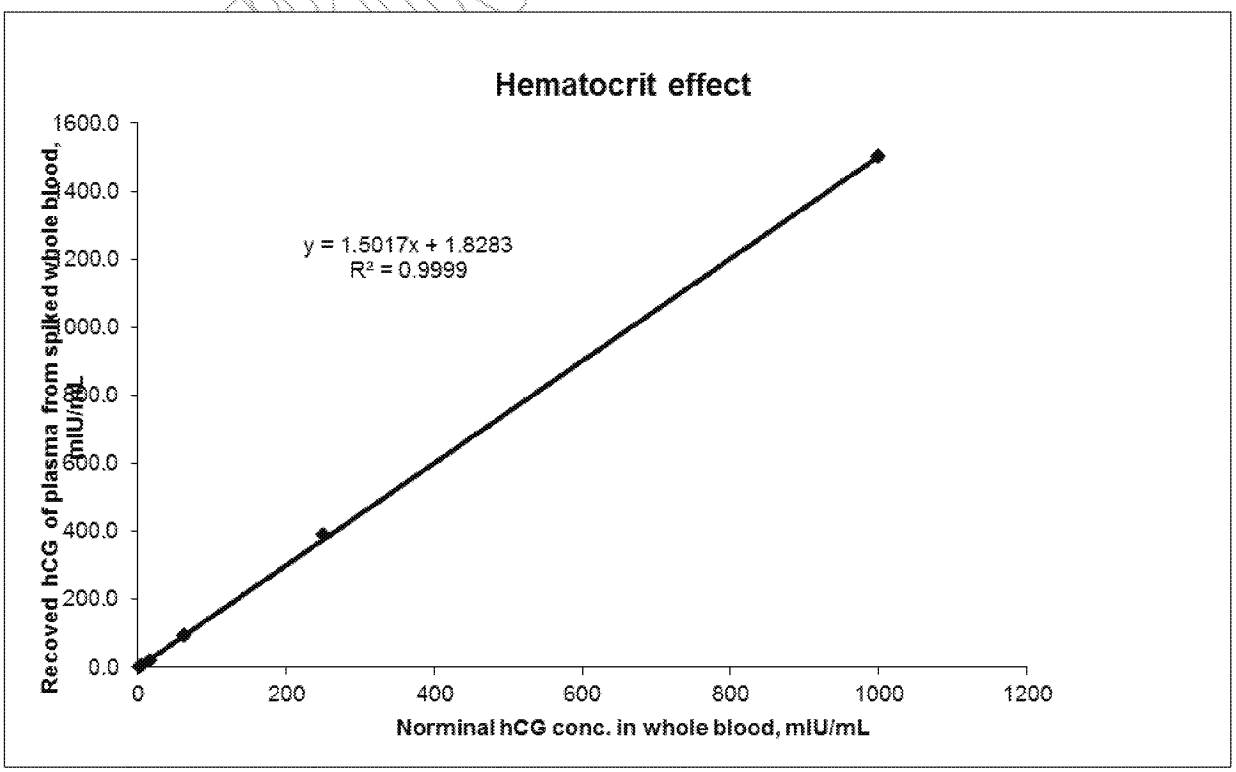
Spiked into Icteric serum

hCG [mIU/ml]		Signal [RLU]			Recovered hCG [mIU/ml]			
Spiked	Norminal	Mean RLU	StDev	%CV	Mean Conc	StDev	%CV	% Recovery
1000.0	1001.6	1322273	207558	16	1002.1	168	17	100
250.0	251.6	203328	10901	5	216.1	8	4	86
62.5	64.1	26442	3054	12	57.7	5	9	90
15.6	17.2	5146	417	8	12.5	1	10	73
3.9	5.5	2315	277	12	4.2	1	19	76
0.0	1.6	1314	115	9	1.6	0	16	
						Ave. % Recovery		85
						Ave. Conc %CV		12

7. Hematocrit Effect

10X hCG calibrators at six levels were spiked into whole blood and tested the spiked whole blood samples and plasma samples from spiked whole blood.





8. Selectivity Test

10X hCG calibrators at 4 levels were spiked into 10 plasma samples: 5 from female donors and 5 from male donors.

#1 Male plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	1786.2	5.6	8.7	71.7
2	3.9	1445.8	3.7	16.4	94.3
3	2.0	1286.6	2.2	21.2	111.2
4	0.0	870.4			
				Ave. Recovery	92.4

#2 Male plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2312.5	8.1	23.4	104.1
2	3.9	1824.5	4.3	7.3	111.3
3	2.0	2500.5	2.5	22.4	126.3
4	0.0	2092.0			
				Ave. Recovery	113.9

#3 Male plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2675.2	11.5	25.0	147.2
2	3.9	1616.9	4.5	12.9	115.7
3	2.0	1363.7	3.3	18.1	167.1
4	0.0	1106.4			
				Ave. Recovery	143.3

#4 Male plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	1874.5	6.2	NA	80.0
2	3.9	1324.4	3.1	14.8	78.9
3	2.0	1127.2	2.2	29.8	111.4
4	0.0	1091.6			
				Ave. Recovery	90.1

#5 Male plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2757.1	12.0	13.8	154.1
2	3.9	1506.0	4.0	21.0	102.8
3	2.0	1389.4	3.4	7.4	173.1
4	0.0	1098.0			

#1 Female plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2016.6	7.1	23.9	90.4
2	3.9	1911.7	6.4	13.0	165.0
3	2.0	1693.6	4.1	9.4	207.4
4	0.0	1300.3			
				Ave. Recovery	154.3

#2 Female plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2748.5	12.0	7.8	153.3
2	3.9	1417.6	3.6	25.1	92.8
3	2.0	1384.4	3.3	26.3	168.7
4	0.0	1118.0			
				Ave. Recovery	138.3

#3 Female plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2734.1	11.9	16.3	152.2
2	3.9	1337.4	3.3	20.9	84.8
3	2.0	1246.3	2.5	16.0	131.0
4	0.0	1000.3			
				Ave. Recovery	123.0

#4 Female plasma

Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2681.7	11.5	12.3	147.3
2	3.9	1472.4	3.7	21.7	95.1
3	2.0	1224.8	2.5	9.1	127.2
4	0.0	1054.5			
				Ave. Recovery	123.2

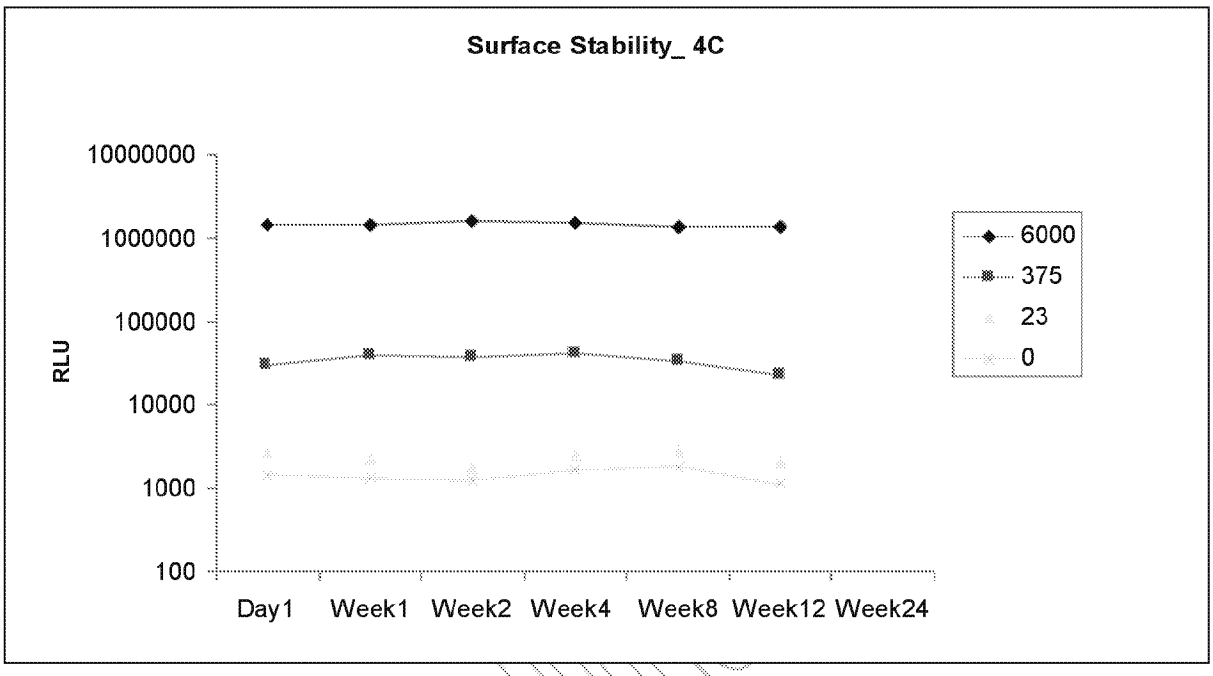
#5 Female plasma

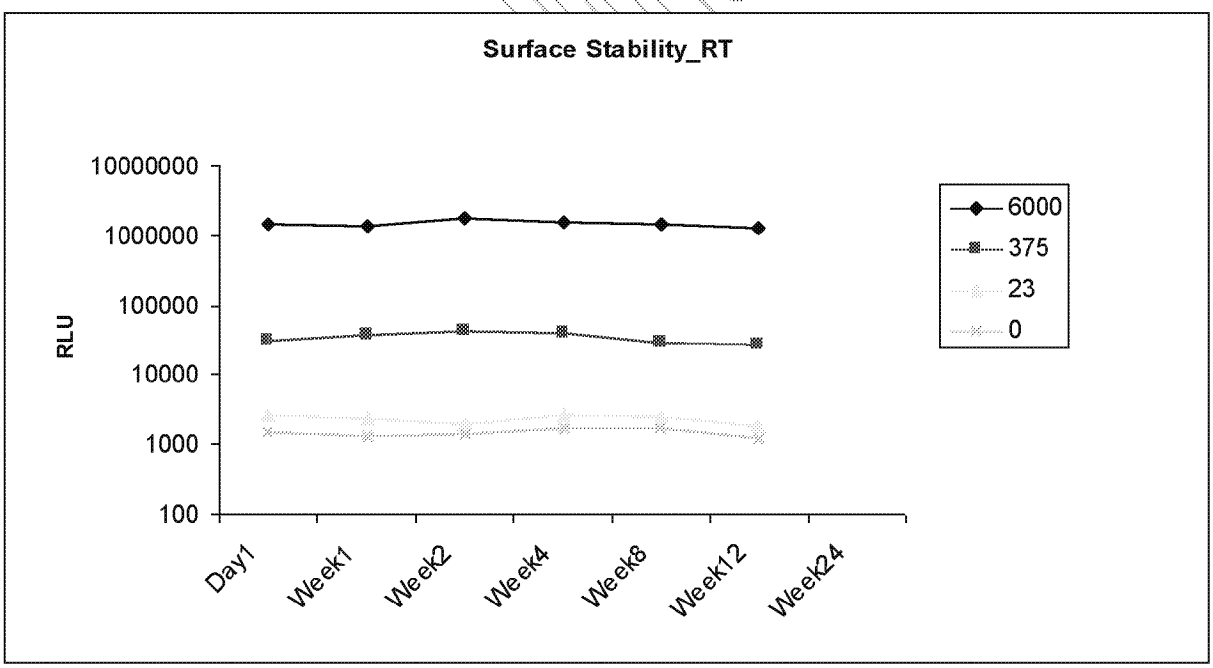
Sample	mIU/ml	MeanValue	Mean Cal conc.	Conc. CV%	% Recovery
1	7.8	2708.4	11.7	11.8	149.7
2	3.9	1488.9	3.8	17.8	96.6
3	2.0	1293.3	2.7	13.6	138.1
4	0.0	1019.1			
				Ave. Recovery	128

9. Stability

Capture antibody surface (stored at both 4C & RT) Stability

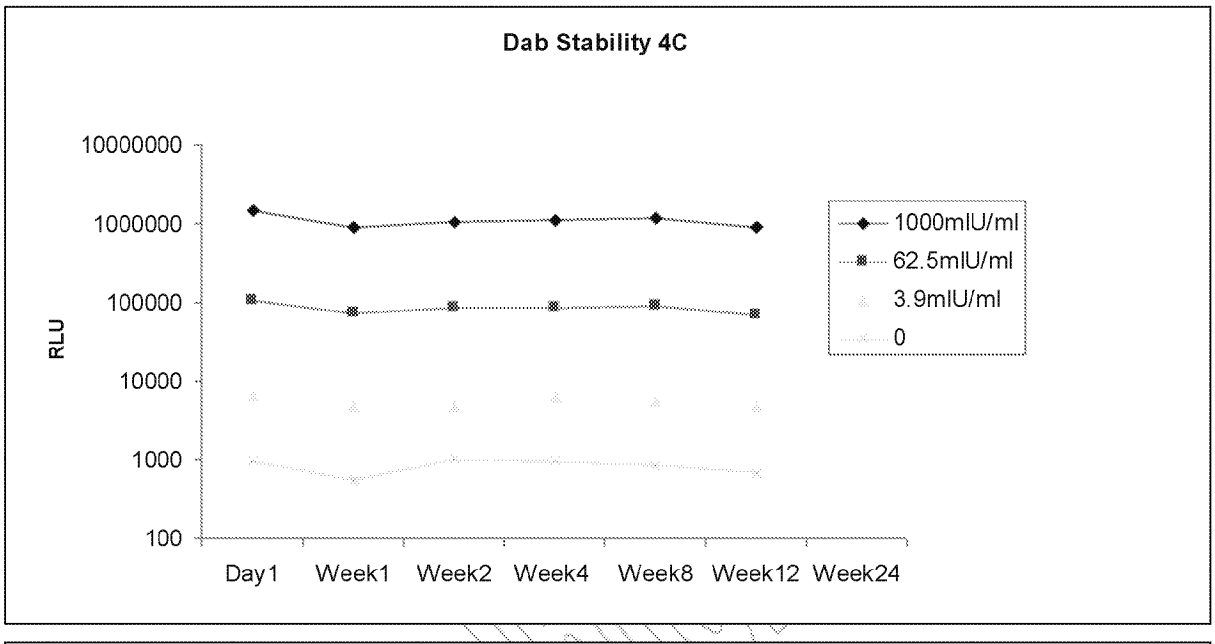
Stability had been tested for a period of 24 weeks for storage at 4°C and room temperature with a 4-point assay buffer curve with an instrument protocol that allows for 10-fold sample dilution. Analyte standards were pre-made for the entire study, aliquoted and flash frozen for single time use. A freshly made working concentration of detection antibody is made for each time point.

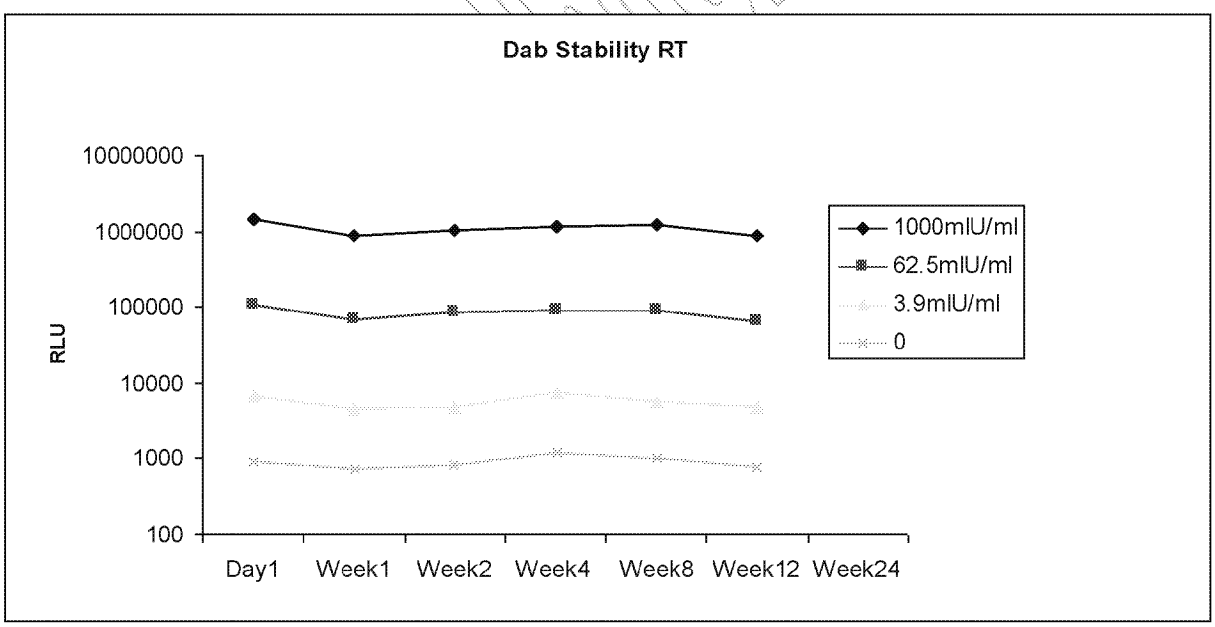




Detection Ab (stored at both 4C & RT) Stability

Detection antibody stability at working concentration had been tested for a period of 24 weeks for storage at 4°C and room temperature in 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.



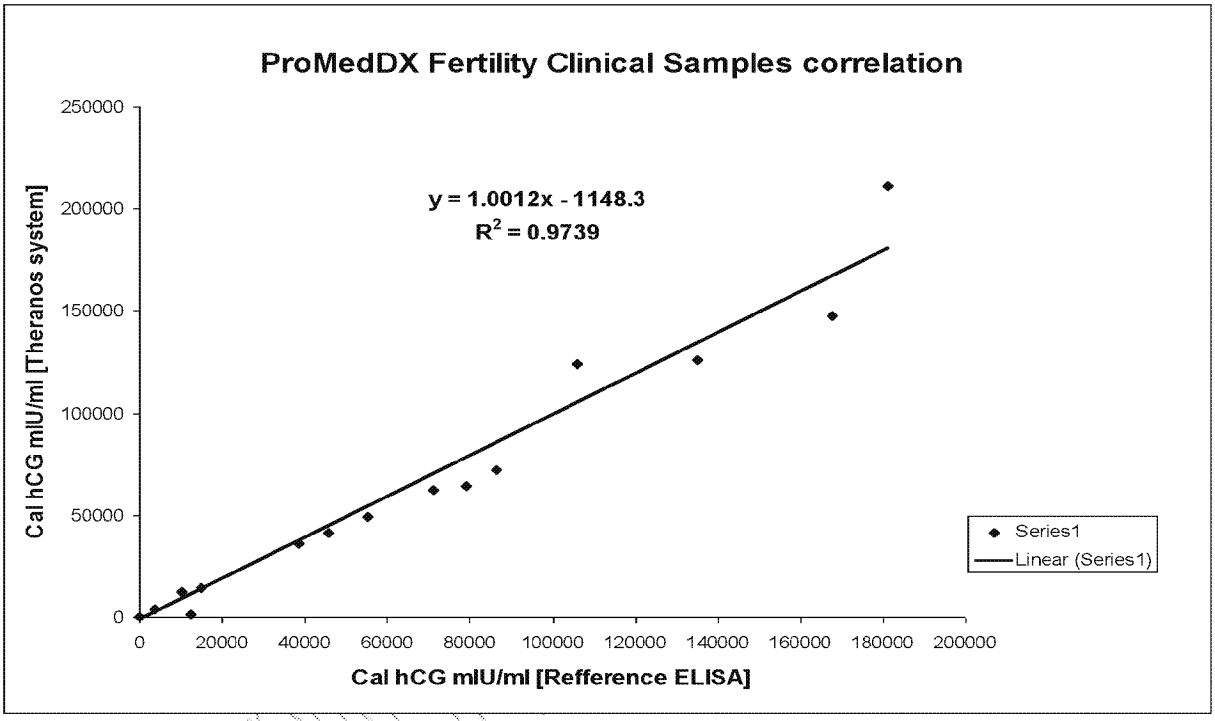


10. Clinical Samples

Thirty-five clinical samples from PromedDx were tested in reference ELISA kit and Theranos assay system for clinical correlation in the Theranos assay system. The correlation was good with R^2 = 0.9739 and slope = 1.0012

Pregnancy Samples
Menstruation samples
Post Menopausal Samples

Sample Theranos#	Theranos system Cal mIU/ml	ELISA Cal mIU/ml
50	62350	71153
51	41666	45797
52	124334	106167
53	12452	10266
54	125766	135050
55	147387	167773
56	211368	181189
57	3926	3663
58	14296	14848
59	49237	55287
60	71920	86263
61	36112	38343
62	64500	79027
63	1608	12163
1	0.193	0.868
2	0.146	0.962
5	0.193	1.092
6	0.251	1.120
7	0.168	0.832
8	0.146	1.270
9	0.251	1.240
12	0.168	1.082
13	0.193	1.258
14	0.251	0.711
15	0.168	0.724
16	0.322	1.126
19	0.108	0.743
20	0.078	1.037
21	0.146	0.901
22	0.751	1.572
26	0.168	1.491
27	0.322	0.903
28	0.146	0.996
80	0.619	1.010
81	0.683	0.467


ProMedDX Fertility Clinical Samples correlation
y = 1.0012x - 1148.3
R2 = 0.9739
Cal hCG mIU/ml [Theranos system]
Cal hCG mIU/ml [Refference ELISA]
0
50000
100000
150000
200000
250000
0
20000
40000
60000
80000
100000
120000
140000
160000
180000
200000
Series1
Linear (Series1)

11. Urine matrix effect

5 point hCG calibrators were spiked into urine samples. The recovery was estimated with plasma calibration curve.

Urine matrix hasno effect on hCG assay

Spiked into plasma

Sample	[hCG], mIU/mL	Mean RLU	Std.Dev.	CV%	Cal. Conc.	% Recovery
1	1000	833121	91322	11	995.1	100
2	250	97420	37682	39	235.7	94
3	62.5	14112	2731	19	68.8	110
4	15.6	2740	395	14	13.9	89
5	3.9	1156	212	18	4.1	105
6	0	861	79	9	2.5	
	S/B	967				

Spiked into urine sample1

Sample	[hCG], mIU/mL	Mean RLU	Std.Dev.	CV%	Cal. Conc.	Recovery
1	1000	756592	58583	8	918.1	92
2	250	102636	8612	8	243.0	97
3	62.5	14336	2002	14	69.6	111
4	15.6	2876	303	11	14.7	95
5	3.9	1391	263	19	5.5	140
6	0	925	132	14	2.8	
	S/B	818				

Spiked into urine sample2

Sample	[hCG], mIU/mL	Mean RLU	Std.Dev.	CV%	Cal. Conc.	Recovery
1	1000	783347	67205	9	944.9	94
2	250	107079	12546	12	249.0	100
3	62.5	12045	1544	13	60.8	97
4	15.6	2786	212	8	14.2	91

5	3.9	1309	160	12	5.0	128
6	0	1040	120	12	3.5	
	S/B	753				

12. Effect of assay time

Protocol test _Effect of assay time with 10Xsample dilution & two step protocol

Summary:

	10/10/10 min		5/5/5 min		2/2/1 min	
hCG mIU/mL	Inter Mean	Inter %CV	Inter Mean	Inter %CV	Inter Mean	Inter %CV
1000	1316780	4.5	595591	13.4	98276	14.4
250	260332	9.2	80310	14.7	12493	14.1
62.5	34605	15.9	10670	13.4	2179	20.5
15.6	6611	9.1	2692	3.3	592	13.9
3.9	3348	9.6	1074	11.4	426	21.2
0	1534	24.3	753	10.7	308	13.9
Std1/B	**858**		**791**		**319**	
Std5/B	**2.2**		**1.4**		**1.4**	

13. Effect of sample dilution with 2/2/1 min protocol

hCG assay range can go as high as 40000mIU/mL with 50X sample dilution of 2/2/1 min protocol. But the assay sensitivity was poor

hCG assay dose responses were saturated after 10000mIU/mL with 20X sample dilution of 2/2/1 min protocol.

hCG assay range can go as high as 10000mIU/mL with 10X sample dilution of 2/2/1 min protocol.

Protocol test _Effect of sample dilution (20X & 50X) with extended assay range (40000 mIU-4 mIU)

Summary:

		Generic2_50x_Coincubation_2-1		Generic2_20x_Coincubation_2-1		Generic2_10x_2-2-1	
Std	hCG mIU/mL	RLU Mean	Inter %CV	RLU Mean	Inter %CV	RLU Mean	Inter %CV
1	40000	926559	12.6	1534897	7.1	NA	
2	20000	622172	10.0	1321984	12.3	NA	
3	10000	301531	13.5	965138	9.5	1017762	9.6
4	4000	111961	7.6	333541	12.6	541489	6.6
5	1000	17923	9.7	56454	15.5	98276	14.4
6	250	2926	8.1	8038	15.0	12493	14.1
7	62.5	679	6.8	1345	10.7	2179	20.5
8	15.6	315	18.5	481	10.9	592	13.9
9	3.9	231	6.2	234	9.3	426	21.2
10	0	210	8.8	205	15.8	308	13.9
	Std1/B	4420.9		7487.7		3302	
	Std8/B	1.5		2.3		2	
	Std9/B	1.1		1.1		1.4