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Estradiol (17 beta-Estradiol) Assay Validation Report

Theranos, Inc.

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

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).

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

The Theranos estradiol assay is designed to detect estradiol in human whole blood, plasma and serum. The assay has a reportable range of 22 – 636 pg/mL.

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

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

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. Interference[TC "Interference" \f C \l "1"]

The assay was tested for interference from other analytes by spiking a low endogenous serum sample with an appropriate concentration of the test substance and using the spiked serum as a calibrator diluent for an estradiol standard curve.

Results:

- Recovery was within 25% of nominal for all spiked levels, no significant interference was observed.

Test Substance	[Test Substance] pg/mL	Nominal [Estradiol] pg/mL	Recovered [Estradiol] pg/mL		
			Mean Conc.	CV %	% Recovery
Estrone	100	494	506	13	102
		372	360	8	97
		250	302	18	121
		189	212	3	112
		128	135	7	106
		6	OORL		
Estriol	500	494	469	32	95
		372	381	14	102
		250	261	10	104
		189	198	3	105
		128	113	9	88
		6	OORL		
Progesterone	10,000	494	487	1	99
		372	316	21	85
		250	268	14	107
		189	199	8	105
		128	120	8	94
		6	OORL		
Cortisol (Hydrocortisone)	2000	494	460	16	93
		372	330	11	89
		250	287	17	115
		189	196	8	104
		128	132	13	103
		6	OORL		

6. Precision: 24 Instruments[TC "Precision: 24 Instruments" \f C \l "1"]

A serum sample was run on 24 different instruments and the inter-instrument/inter-cartridge CVs were determined.

Results:

- Total signal CVs over 24 instruments with internal duplicates were 9%
- Total concentration CVs across 24 instruments (precision) were 13% and recovery (accuracy) was 92%

Instrument	Signal (RLU)		Conc. pg/mL
	Mean RLU	Difference from Mean	
1	9287	-5	74
2	9523	-2	71
3	9991	2	66
4	10160	4	64
5	9397	-4	72
6	10383	7	62
7	10275	5	63
8	10351	6	62
9	8432	-14	84
10	8334	-15	86
11	9041	-7	77
12	9457	-3	72
13	10687	10	59
14	10984	13	57
15	10704	10	59
16	10823	11	58
17	10125	4	65
18	10222	5	64
19	10168	4	64
20	10452	7	61
21	8832	-9	79
22	8365	-14	85
23	8425	-14	84
24	9562	-2	71

Inter-Instrument Signal CVs		
Mean RLU	StDev	CV%
9749	835	9

Inter-Instrument Concentration CVs			
Mean Conc. pg/mL	StDev	CV%	% Recovery
69	9	13	92

7. Three Day Precision and Accuracy[TC "Three Day Precision and Accuracy" \f C \l "1"]

A 12 point calibration curve was run with triplicate cartridges on 3 days and a calibration equation was created from the 3 day data set. The calibration curve was used to back-calculate the concentration for each data point, to generate a 3 day accuracy and precision test. In addition, 3 QC levels were run in triplicate each day and the accuracy and precision at high, medium and low QC levels were determined.

Results:

- At LLOQ of 22 pg/mL, accuracy was 98% and precision was 12%
- At ULOQ of 636 pg/mL, accuracy was 89% and precision was 14%
- At High QC level of 479 pg/mL, accuracy was 95% and precision was 8%
- At Medium QC level of 93 pg/mL, accuracy was 99% and precision was 14%
- At Low QC level of 45 pg/mL, accuracy was 90% and precision was 11%

Calibration Data for 9 Replicates Per Point

Model Type	LogLin 4PL				
Model Equation	log10(RLU) = b1+(b2-b1)/(1+(Conc/b3)^b4				

Calibration Equation	If RLU <= SignalMax & RLU >= SignalMin Then
	Conc = b3 * (((b2 - b1) / (log10(RLU) - b1)) - 1) ^ (1 / b4)
	End If				

LLOQ	21.75	pg/mL	Model Parameters		SE
ULOQ	636.00	pg/mL	b1	3.059	0.050
desired LLOQ	21.75	pg/mL	b2	4.398	0.017
desired ULOQ	636.00	pg/mL	b3	127.698	8.655
LLOD	Not Reported	pg/mL	b4	1.065	0.069
LLOQ accuracy	98	%	b5		
LLOQ precision	12.4	%			
Average Residuals	7	%			
Error in prediction: Best case	12	%			
Error in prediction: Expected	12	%			
Signal Min	1736	RLU			
Signal Max	17980	RLU			
Outliers detected based on within-standard variance and manual detection	Outliers				
	Conc	RLU			
Statistically Detected ■	None				
Manually Selected ■	None				

Three Day Precision and Accuracy

Nominal [Estradiol] pg/mL	Cartridge	Recovered [Estradiol] pg/mL					
		Day 1	Day 2	Day 3	Mean Conc.	CV %	% Recovery
636.00	1	688	599	495	566	14	89
	2	507	603	OORH			
	3	OORH	613	459			
321.00	1	316	392	417	349	19	109
	2	439	320	258			
	3	279	317	400			
163.50	1	166	165	163	158	9	97
	2	156	142	131			
	3	179	162	157			
132.00	1	117	124	124	133	11	100
	2	117	126	137			
	3	145	142	162			
100.50	1	129	117	101	106	11	106
	2	91	103	112			
	3	107	95	100			
84.75	1	102	90	77	88	9	103
	2	81	89	92			
	3	95	84	79			
69.00	1	68	59	58	68	11	99
	2	69	81	76			
	3	67	66	69			
53.25	1	53	47	47	54	11	101
	2	63	59	59			
	3	49	52	54			
37.50	1	32	36	34	39	15	105
	2	38	39	41			
	3	52	43	39			
21.75	1	OORL	19	21	22	8	102
	2	24	25	23			
	3	22	23	21			
12.25	1	OORL	OORL	OORL	OORL		
	2	OORL	OORL	OORL			
	3	OORL	OORL	OORL			
6.00	1	OORL	OORL	OORL	OORL		
	2	OORL	OORL	OORL			
	3	OORL	OORL	OORL			

QC Levels for 3 Day Precision and Accuracy

Nominal [Estradiol] pg/mL	Cartridge	Recovered [Estradiol] pg/mL					
		Day 1	Day 2	Day 3	Mean Conc.	CV %	% Recovery
478.50	1	522	481	448	455	8	95
	2	402	426	485			
	3	440	444	444			
92.63	1	114	104	72	92	14	99
	2	104	91	85			
	3	90	81	87			
45.38	1	36	37	37	41	11	90
	2	40	38	44			
	3	44	48	44			

8. Dilution Linearity[TC "Dilution Linearity" \f C \l "1"]

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:

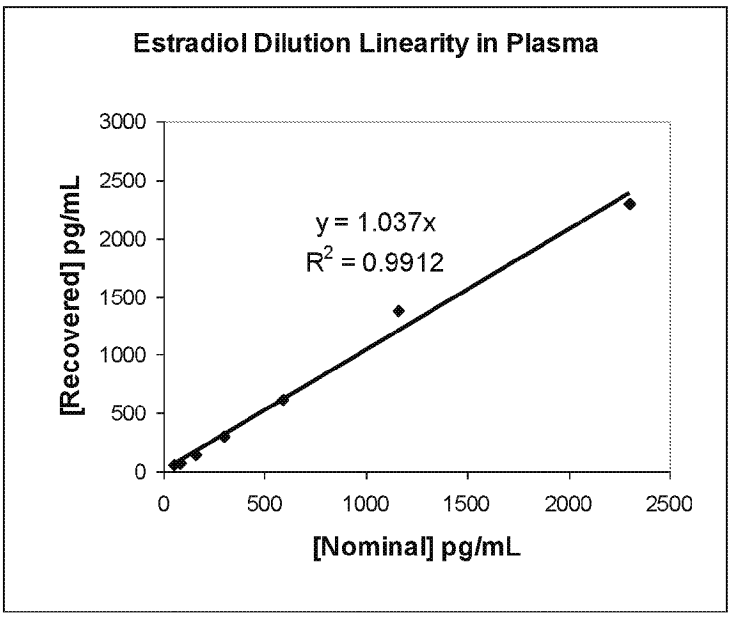
- Recovery was consistently within 25% of nominal over the range of the dilution series in serum and plasma.

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	-	-	-

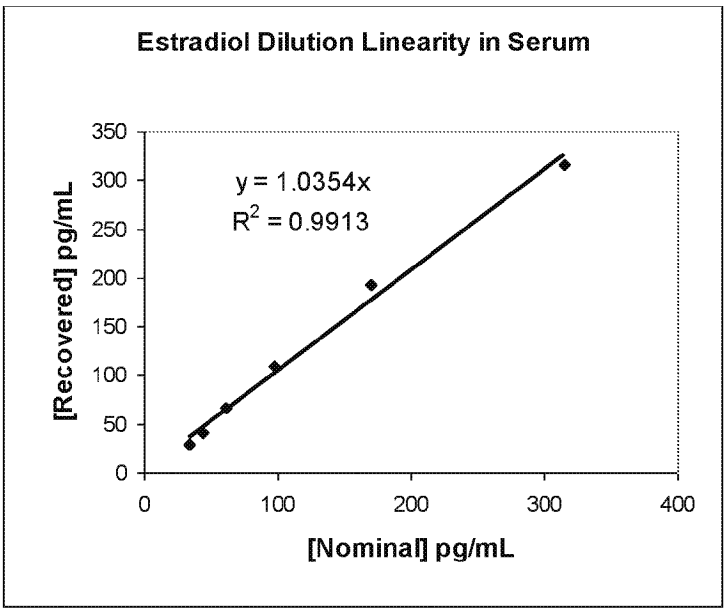
OORL: Out of range low


Estradiol Dilution Linearity in Plasma
[Recovered] pg/mL
[Nominal] pg/mL
y = 1.037x
R2 = 0.9912
0
500
1000
1500
2000
2500
3000

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



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

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

Results:

1. All samples showed recovery within 25% of nominal for at least 4 of 5 spiked levels, meeting the acceptance criteria for whole blood spike recovery.

Sample	[Estradiol] pg/mL		Recovered [Estradiol] pg/mL			
	Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
A	488	505	413	48	12	82
	366	383	323	27	8	84
	244	261	284	10	4	109
	183	200	227	20	9	113
	122	139	167	32	19	120
	0	17	17	1	3	
B	488	527	543	154	28	103
	366	405	368	34	9	91
	244	283	290	12	4	102
	183	222	276	1	1	124
	122	161	224	7	3	139
	0	39	39	6	16	
C	488	509	462	88	19	91
	366	387	380	38	10	98
	244	265	284	22	8	107
	183	204	206	25	12	101
	122	143	184	39	21	129
	0	21	21			

10. Hematocrit Effect[TC "Hematocrit Effect" \f C \l "1"]

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

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

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

A plasma sample was spiked with 6 levels of estradiol and the recovery was calculated.

Results:

- Recovery was within 25% of nominal for all spike levels.

Matrix	[Estradiol] pg/mL		Recovered [Estradiol] pg/mL		
	Spiked	Nominal	Mean Conc.	CV %	% Recovery
Normal Plasma	488	534	541	18	101
W070510115597	366	412	513	11	124
	244	290	340	14	117
	183	229	276	12	120
	122	168	194	5	115
	0	46	46	2	

12. Selectivity [TC "Selectivity" \f C \l "1"]

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

Results:

1. 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

13. Matrix Effects

The Theranos estradiol assay is calibrated using pooled serum standards. The effect of other matrixes including lipemic serum, hemolyzed serum and icteric serum was tested.

Results:

- Extremely hemolyzed samples may interfere with the assay.
- Extremely icteric samples may cause over-recovery.
- Lipemic samples show no interference in this assay.

Matrix	[Estradiol] pg/mL		Recovered [Estradiol] pg/mL		
	Spiked	Nominal	Mean Conc.	CV %	% Recovery
Hemolyzed Serum	488	531	396	14	75
ProMedDx 11213623	366	409	290	4	71
	244	287	230	14	80
	183	226	186	10	82
	122	165	148	20	90
	0	43	43	17	-
Icteric Serum	488	550	OORH	-	-
Vital SFB8310	366	428	576	27	134
	244	306	336	27	110
	183	245	328	12	134
	122	184	294	7	159
	0	62	62	10	-
Lipemic Serum	488	534	563	-	105
ProMedDx 11121161	366	412	516	6	125
	244	290	292	6	101
	183	229	242	9	106
	122	168	155	27	92
	0	46	46	28	-

14. Extended Range[TC "Extended Range" \f C \l "1"]

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

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

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 and this calibration was fit to a Log-Lin 4 Parameter Logistical curve, 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

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

Clinical serum samples obtained from ProMedDx were run on the Theranos System and on reference methods;

- Alpco Estradiol EIA Catalog # 110-ESTHU-E01,
- Alpco Ultrasensitive Estradiol ELISA Catalog # EIA-4399
- Invitrogen Estradiol ELISA Catalog # KAQ0621.

Samples that lay out of range for the Alpco Ultrasensitive kit were prediluted as appropriate in the manufacturer-provided sample diluent.

Results:

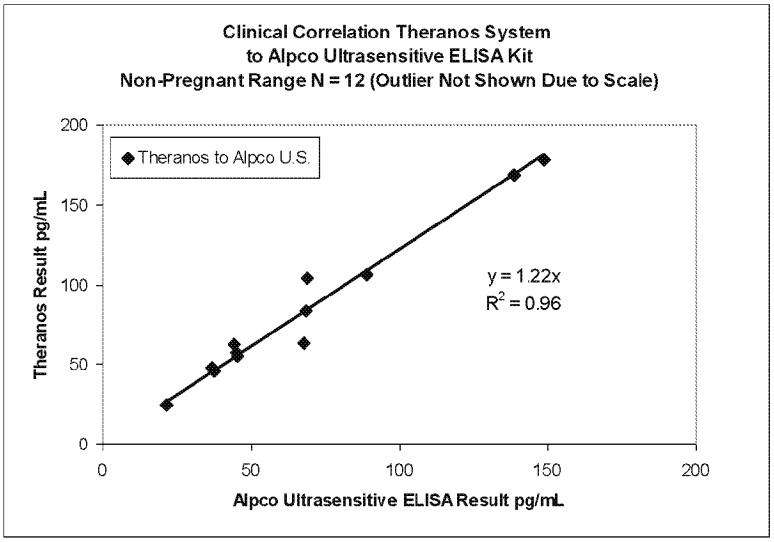
- Theranos System results correlated well with the commercial methods tested
- Correlation of the Theranos System was best with the Alpco Ultrasensitive ELISA with the exception of sample #82 (see additional note below).
- In the non-pregnant range, correlation of Theranos results to each reference method was better than the correlation among the reference methods.

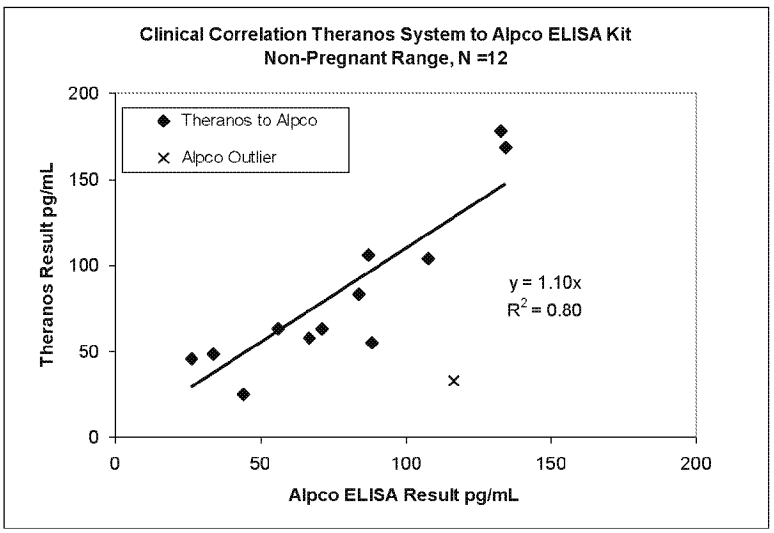
Note about sample #82: Postmenopausal women are expected to have < 50 pg/mL estradiol (14), so the Alpco Regular and Ultrasensitive Kit results appear to be discrepant, whereas Invitrogen and Theranos results match closely and are consistent with the expected levels of estradiol for postmenopausal women.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

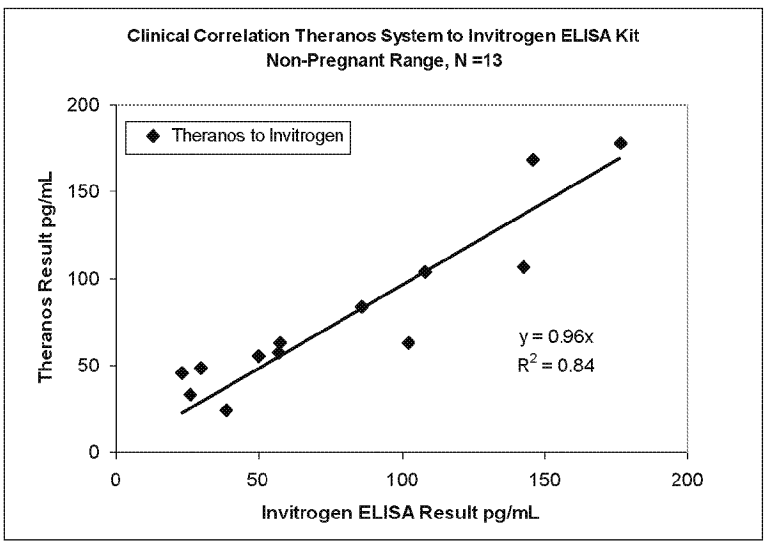
Clinical Sample Results

Sample #	Set	Day/ Week	Reference Result pg/mL			Theranos Result pg/mL		
			Invitrogen	Alpco	Alpco U.S.	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	69	83	15	18
13	Menstruation	13	142	87	89	106	8	8
15	Menstruation	15	176	133	149	178	34	19
21	Menstruation	21	146	134	139	168	36	22
26	Menstruation	26	108	108	69	104	4	4
28	Menstruation	28	58	56	68	63	6	9
51	Pregnant	5	337	210	179	177	38	21
52	Pregnant	7	440	689	520	OORH		
53	Pregnant	5	271	182	113	139	11	8
55	Pregnant	9	333	242	162	142	23	16

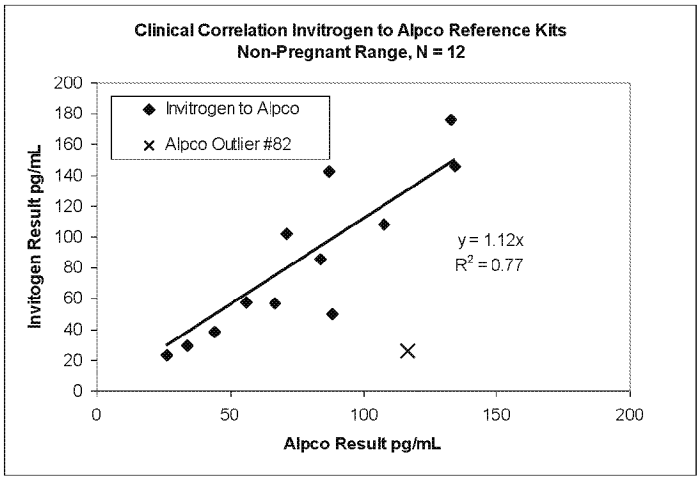
OORH = Out of Range High

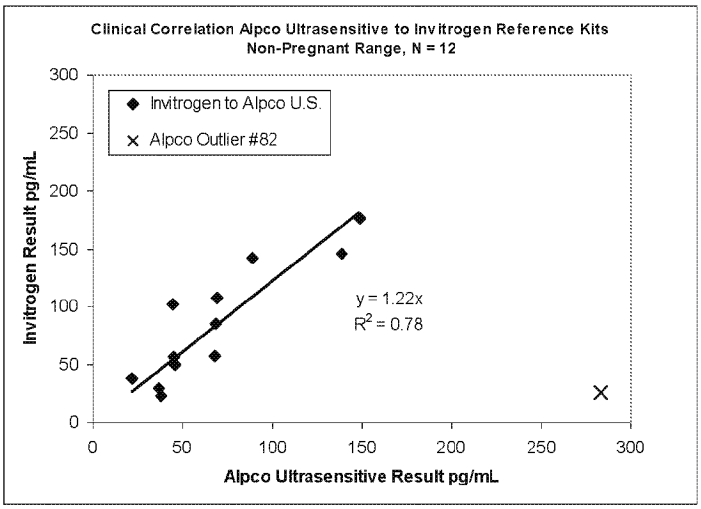

Clinical Correlation Theranos System
to Alpco Ultrasensitive ELISA Kit
Non-Pregnant Range N = 12 (Outlier Not Shown Due to Scale)
Theranos to Alpco U.S.
y = 1.22x
R2 = 0.96
Theranos Result pg/mL
Alpco Ultrasensitive ELISA Result pg/mL
0
50
100
150
200

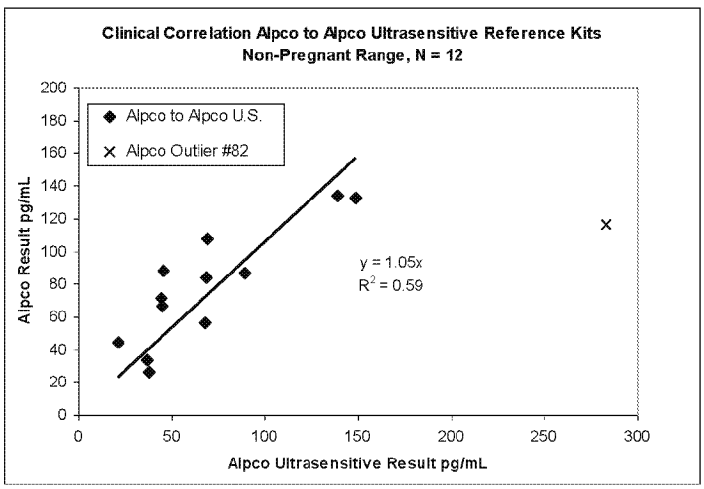

Clinical Correlation Theranos System to Alpco ELISA Kit
Non-Pregnant Range, N =12
Theranos to Alpco
Alpco Outlier
y = 1.10x
R2 = 0.80
Theranos Result pg/mL
Alpco ELISA Result pg/mL
0
50
100
150
200


Clinical Correlation Theranos System to Invitrogen ELISA Kit
Non-Pregnant Range, N =13
Theranos to Invitrogen
y = 0.96x
R2 = 0.84
Theranos Result pg/mL
Invitrogen ELISA Result pg/mL
0
50
100
150
200

Correlation among the three reference methods in the non-pregnant range:



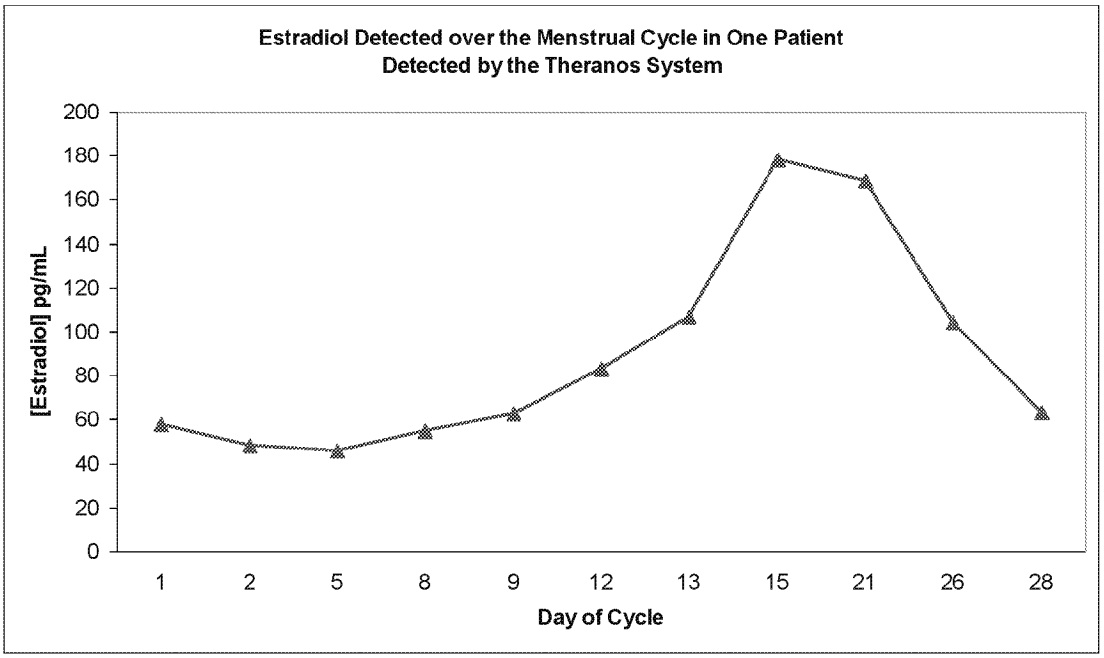




As part of the assay clinical validation we measured the levels of estradiol over a menstrual cycle using serum samples from a single individual from ProMedDx.

Results:

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



18. Stability[TC "Stability" \f C \l "1"]

Stability testing of the reagents was begun using the early stages of assay development. 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:

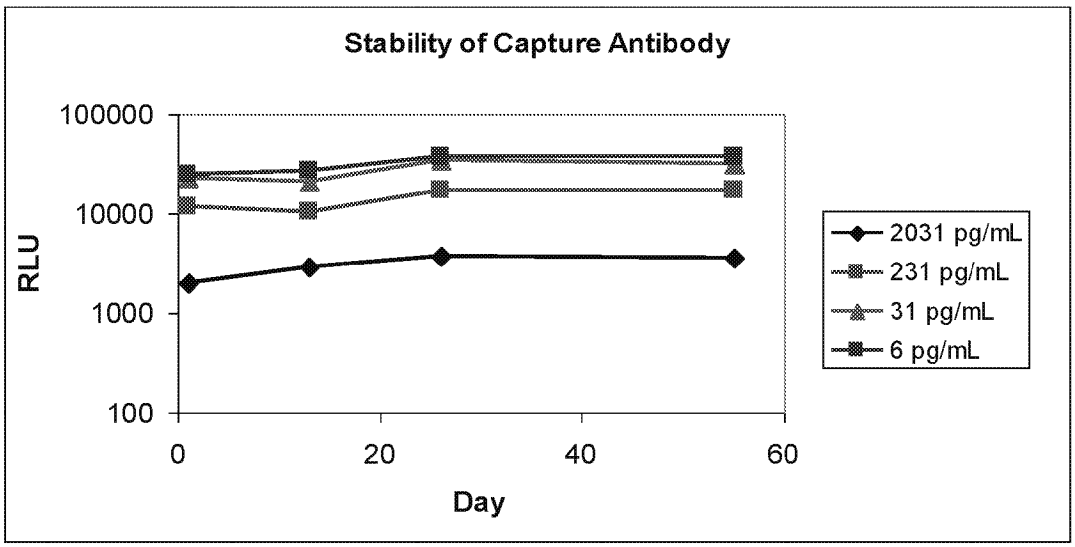
- In these initial tests, the reagents are showing good stability.

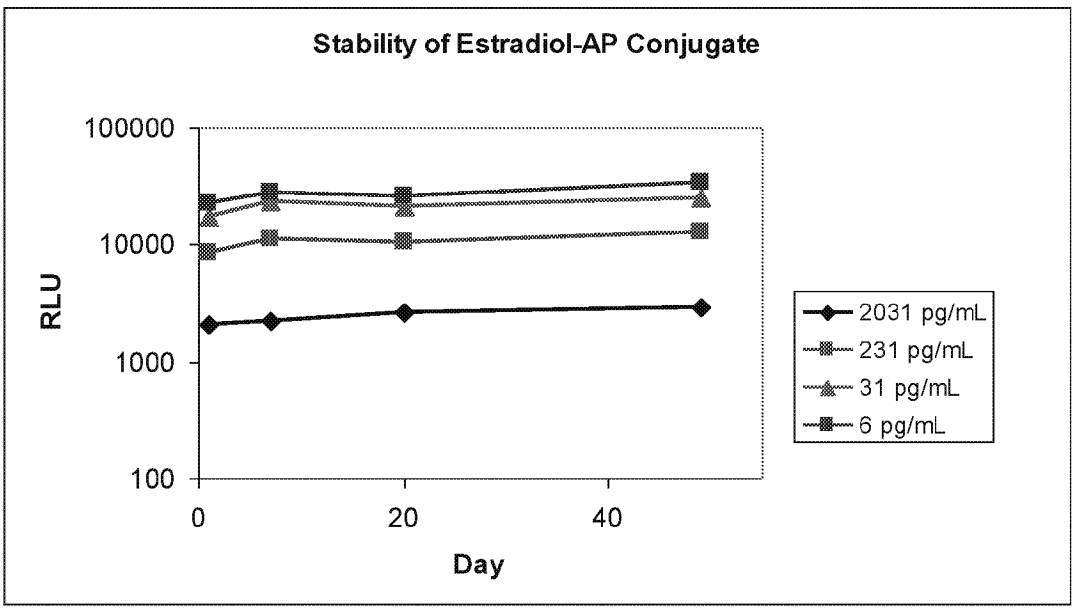
Stability of Capture Antibody

			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 Conjugate

			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 Capture Antibody
RLU
100000
10000
1000
100
0
20
40
60
Day
2031 pg/mL
231 pg/mL
31 pg/mL
6 pg/mL


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

References[TC "References" \f C \l "1"]

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