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Progesterone

Assay Development Report

Theranos, Inc.

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1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications[TC "Assay Specifications" \f C \l "3"]

The assay is designed to detect progesterone in human whole blood, plasma, and serum. The assay has a reportable range of 100 – 0.3 ng/mL, in the above mentioned matrixes.

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

The following commercial ELISA kits has been used in house as predicate methods:
Progesterone ELISA IBL Catalog Number: 55R-RE52231

1.1.2 Materials and Methods[TC "Materials and Methods" \f C \l "1"]

This assay is a sandwich ELISA.

2 ASSAY DEVELOPMENT[TC "ASSAY OPTIMIZATION" \F C \L "2"]

2.1 Cross Reactivity

Cross reactivity with estradiol was tested up to 30 ng/mL. No cross reactivity was observed.

Test Solutions	Test Substance Level (ng/mL)	Mean Calculated Concentration (ng/mL)	Assay Result
Progesterone	0.000	0.01	OORL
Estradiol	30.0	0.00	OORL
	6.0	0.00	OORL
	1.2	0.01	OORL
	0.2	0.01	OORL
	0.0	0.01	OORL
	0.0	0.01	OORL

OORL = Out of Range Low

2.2 Whole Blood Screen and Hematocrit Effect

Normal male and female whole blood and plasma samples were analyzed in the Theranos System. Results were not significantly different when measured in whole blood or the plasma obtained from whole blood. All results were within the expected range for non-pregnant females and male donors.

Sample #	Whole Blood Result, ng/mL	Plasma Result, ng/mL	% Recovery in Plasma vs WB
1	0.27	0.34	125
2	1.47	1.50	102
3	0.60	0.53	88
4	1.43	1.56	109
2	0.44	0.49	112
3	0.78	0.52	66
1	0.46	0.42	92
2	0.37	0.35	95
3	0.56	0.59	107
4	0.15	0.17	118

2.3 Inter-Lot Accuracy and Precision

For precision testing, an 8 point standard curve of serum calibrators was run for three lots of reagents.

Summary

Average Inter-Lot Precision	13.8 %
Average Intra-Lot Accuracy	101 %

Table [SEQ Table * ARABIC]: Inter-Lot Precision and Accuracy

[Progesterone] ng/mL	Mean Conc (ng/mL)	CV %	% Recovery
62.3	72.5	6.5	116
48.5	47.2	12.2	97
37.1	31.8	13	86
11.3	11.5	14.4	102
4.4	5.6	11.9	127
1.5	1.4	12.3	93
0.8	0.7	14.1	88
0.1	0.1	26.1	100
Average		13.8	101

2.4 Inter-Instrument Accuracy and Precision

One serum solution in the mid range of the assay (25.1 ng/mL) was assayed in a total of 24 cartridges on 24 different instruments to determine the accuracy and precision.

Summary

Inter-Cartridge/Instrument Precision	7.0 %
Inter-Cartridge/Instrument Accuracy	95%

Table [SEQ Table * ARABIC]: Inter-Cartridge Accuracy and Precision

Mean Calculated Concentration (ng/mL)	STDev	CV %
23.3	1.6	7.0

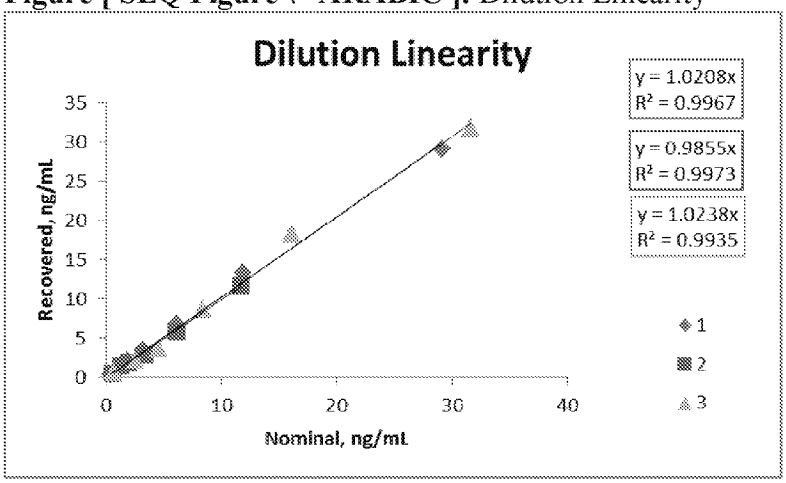
2.5 Dilution Linearity

For each of the 3 tests, two clinical samples- one high and one low – were mixed together to test for dilution linearity. The concentrations of the serial dilutions were calculated based on the volume ratios of the low and high sample used to create them and the nominal concentrations of the low and high samples. Samples showed linear recovery along the reportable assay range.

Table [SEQ Table * ARABIC]: Dilution Linearity

Sample combo	Nominal, ng/ml	Recovered, ng/ml	% Recovery
1	29.1	29.12	100
	11.9	13.25	112
	6.1	6.74	110
	3.3	3.48	107
	1.8	2.09	115
	0.4	0.37	
2	11.71	11.71	100
	6.13	5.86	96
	3.34	2.97	89
	1.95	1.85	95
	1.25	1.49	119
	0.55	0.55	
3	31.67	31.67	100
	16.15	18.29	113
	8.38	8.68	103
	4.5	3.66	81
	2.56	2.21	86
	0.62	0.62	

Figure [SEQ Figure * ARABIC]: Dilution Linearity



2.6 Plasma Spike Recovery

Spike recovery in low endogenous (male) plasma was tested. Recovery was good across the range.

Spiked [Progesterone] ng/mL	Recovered, ng/mL	Concentration % CV	% Recovery
54.4	51.3	12.9	94
25.3	24.8	1.3	98
8.4	7.8	13.3	93
2.3	2.2	15.1	96
0.0	OORL	-	-

2.7 Interfering Matrixes

The assay was tested for matrix effects withlow endogenous (male) lipemic and hemolyzed sera. Based on these results, lipemic samples do not appear to show interference but hemolyzed samples may give lower readings in the Theranos progesterone assay.

Table [SEQ Table * ARABIC]: Spike Recovery in Normal Serum (Control)

Spiked, ng/mL	Recovered, ng/mL	CV %	% Recovery
54.4	44.9	7.5	83
25.3	23.6	5.9	93
8.4	7.4	2.2	88
0	0	14.5	-

Table [SEQ Table * ARABIC]: Spike Recovery in Lipemic Serum

Spiked, ng/mL	Recovered, ng/mL	CV %	% Recovery
54.4	50.8	8.8	94
25.3	26.3	6.5	104
8.4	8.3	9.7	98
0	0	13.2	-

Table [SEQ Table * ARABIC]: Spike Recovery in Hemolyzed Serum

Spiked, ng/mL	Recovered, ng/mL	CV %	% Recovery
54.4	36.1	7.8	66
25.3	18.0	4.7	71
8.4	6.1	11.2	72
0	0	16.2	-

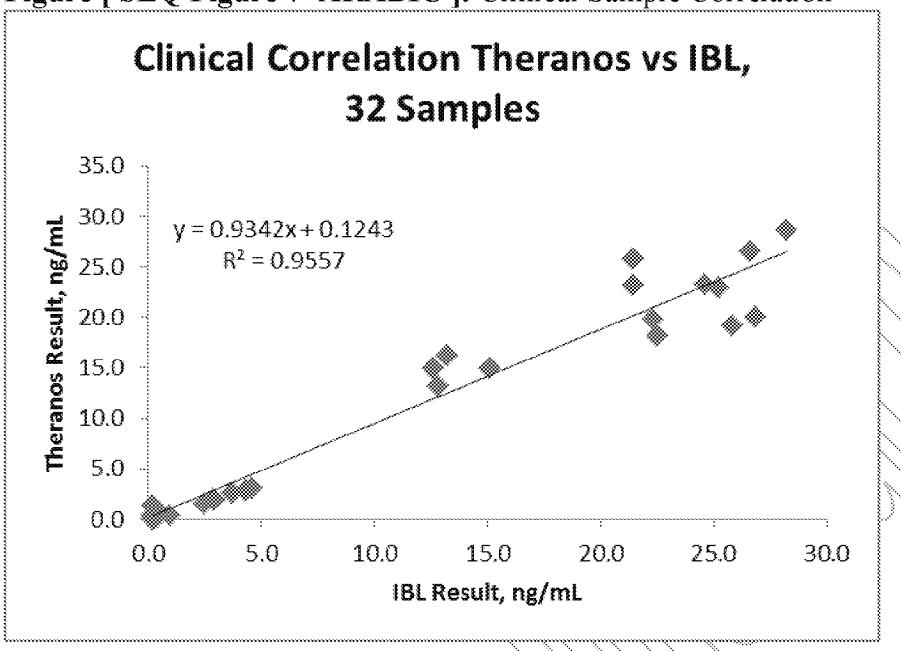
2.8 Clinical Sample Correlation

Clinical pregnancy and normal male serum samples obtained from BioReclamation and ProMedDX were run in the Theranos System and the IBL ELISA. For a total of 32 samples across the range of the assay, correlation was excellent.

Table [SEQ Table * ARABIC]: Clinical Sample Correlation

Sample #	IBL Result, ng/mL	Theranos Result, ng/mL
3	22.5	18.2
40	22.3	19.8
41	26.8	20
47	25.8	19.2
48	24.6	23.3
M2	0.3	0.4
M3	0.4	0.8
1	21.4	25.8
4	15.1	14.9
5	26.6	26.5
6	12.8	13.2
44	25.2	22.9
45	13.2	16.2
46	12.6	14.9
M37	0.3	1.1
M38	0.2	1.4
M39	0.2	0
M1	0.3	0.5
13	0.2	0.1
15	0.3	0.1
16	0.4	0.2
19	2.5	1.5
20	2.9	2
21	4.6	3.1
22	4.3	2.9
26	3.7	2.6
27	2.9	1.8
28	1	0.4
51	28.2	28.6
56	21.4	23.1
80	0.2	0.3
82	0.2	0.3

Figure [SEQ Figure * ARABIC]: Clinical Sample Correlation



2.9 Stability

Stability of reagents is being monitored.

3 CONCLUSION

We have successfully developed an immunoassay to detect Progesterone in human serum and plasma.