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Phencyclidine (PCP) Assay Development Report

Theranos, Inc.

Nov 20, 2012

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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"]

This assay is designed to detect phencyclidine (PCP) in human urine, serum, plasma, or whole blood. The assay has a reportable range of 5 to 500 ng/mL.

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

The following commercial methods were used as comparison methods:

- Bioquant Cat #BQ 208-096 PCP Direct ELISA Kit (urine, whole blood, plasma but directions are specified only for urine)
- DRG Cat#RAP-2833 QuickScreen One Step Phencyclidine Screening Test (urine only)

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

An anti-sheep secondary antibody followed by a primary sheep anti-PCP antibody are coated on the capture surface. The sample is diluted and combined with an enzyme labeled PCP conjugate. This mixture is incubated on the capture surface for 5 minutes. After the incubation, the surface is washed and substrate is incubated on the surface for 5 minutes, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #
Phencyclidine Hydrochloride (CAS 956-90-1)	Sigma	P3029
Alkaline Phosphatase Substrate	Theranos	T-ALKP-SB01
Low BSA Blocking Buffer (0.03% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G
Carbonate-bicarbonate buffer	Sigma	C3041
Sheep PAb Anti-PCP (PCP(m/p)-BSA)	Randox	PAS9716
PCP-Alk Phos Conjugate	Theranos	JX-412-79-2

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

2.1 Antibody-Conjugate Binding Screen (MTP) [TC "Detection Antibody Conjugate Verification" \f C \l "1"]

The anti-PCP antibodies were coated on a 384 well microtitre plate (MTP) at 10, 1, 0.1 and 0 ug/mL and tested for binding to a constant concentration of the PCP-HRPs conjugates from Randox and Calbioreagents and the PCP-AP conjugates from YJ Biosciences and an in-house conjugate.

Table [SEQ Table * ARABIC]: Antibody Information

Antibody #	Vendor	Catalog #	Clone
1	Randox	PAS9714	Sheep PAb
2	Randox	PAS9715	Sheep PAb
3	Randox	PAS9716	Sheep PAb
4	Randox	PAS9717	Sheep PAb
5	US Biological	P3380-10A	9L533
6	US Biological	P3380-10	8.F.225A
7	US Biological	P3380-11D	Sheep PAb
8	US Biological	P3380-11E	10F279
9	Calbioreagents	M326	Not reported

Table [SEQ Table * ARABIC]: Conjugate Information

Conjugate #	Vendor	Catalog #	Type
1	Randox	HRP9325	PCP(cyclohexyl,4)-HRP
2	Randox	HRP9326	PCP(piperidyl,4)-HRP
3	Calbioreagents	C071	PCP-HRP
4	YJ Biosciences	HP2110A	PCP(p)-AP
5	Theranos	JX-412-79-2	PCP-acid-AP

Table [SEQ Table * ARABIC]: Antibody-Conjugate Binding Screen with HRP Conjugates

Conjugate #:		1			2			3		
Ab #	[CAb] ug/mL	Mean RLU	CV %	Mod.	Mean RLU	CV %	Mod.	Mean RLU	CV %	Mod.
1	10	288948	5.7	218	1290879	3.8	1399	24051	17.8	3
	1	42947	0.4	32	228733	2.6	248	12699	8.1	2
	0.1	6330	9.8	5	30223	6.0	33	9195	9.0	1
	0	1325	2.3		923	19.2		7516	37.3	
2	10	1878	17.3	1	853	39.4	1	10932	26.0	1
	1	1520	12.2	1	762	22.5	1	8354	2.6	1
	0.1	1671	15.7	1	853	39.0	1	9115	30.9	1
	0	2153	4.5		1041	28.8		9179	4.1	
3	10	2007	2.2	1	1244	12.0	1	13809	42.0	0
	1	1986	2.9	1	982	3.7	1	9367	19.3	0
	0.1	1486	3.3	1	1389	1.2	1	13695	56.0	0
	0	1919	3.4		1774	6.8		41592	85.9	
4	10	13658	14.4	14	15670	11.3	14	22013	3.2	4
	1	1893	8.7	2	1966	13.4	2	17788	19.5	3
	0.1	935	4.1	1	1078	22.6	1	10444	5.8	2
	0	958	6.5		1143	6.9		6133	1.8	
5	10	2904	0.1	2	14211	13.0	10	15059	24.9	1
	1	1330	11.2	1	1688	7.2	1	8587	24.9	1
	0.1	1091	8.1	1	1293	24.6	1	9829	6.6	1
	0	1604	4.9		1389	3.8		10492	39.2	
6	10	3193	1.6	2	16044	4.2	10	17991	9.7	1
	1	1313	6.7	1	2076	0.6	1	10176	30.4	0
	0.1	2738	83.6	2	1545	17.1	1	11219	1.9	1
	0	1454	5.4		1641	11.2		21829	26.4	
7	10	888	18.6	1	1728	30.5	1	19294	8.1	2
	1	737	13.1	1	1308	26.6	1	18739	22.8	2
	0.1	461	17.5	1	1300	23.1	1	12988	35.8	1
	0	753	21.7		1630	24.8		11418	26.9	
8	10	29623	16.7	23	2325	17.4	1	9789	1.3	0
	1	3454	5.8	3	1845	11.3	1	10631	19.8	1
	0.1	923	17.0	1	1743	8.5	1	19555	53.9	1
	0	1297	18.2		1799	11.2		20836	58.6	
9	10	1179	13.7	1	2750	3.4	2	28593	10.9	1
	1	1501	2.7	1	1486	25.2	1	13811	8.8	1
	0.1	1145	8.1	1	1479	16.2	1	20364	65.5	1
	0	1761	3.5		1501	3.2		20331	21.1	

Table [SEQ Table * ARABIC]: Antibody-Conjugate Binding Screen with AP Conjugates

Conjugate #:		4			5		
Ab #	**[CAb] ug/mL**	**Mean RLU**	**CV %**	**Mod.**	**Mean RLU**	**CV %**	**Mod.**
1	10	1117096	0.6	1481	1978050	1.0	501
	1	72631	23.8	96	914301	12.6	231
	0.1	3733	18.4	5	76824	47.4	19
	0	754	0.0		3952	9.8	
2	10	658210	10.2	1027	1138605	0.4	178
	1	20505	15.8	32	108439	47.4	17
	0.1	2001	8.2	3	13901	26.5	2
	0	641	15.2		6394	52.5	
3	10	531873	5.1	948	1145801	1.1	263
	1	14987	11.5	27	81685	9.6	19
	0.1	2591	63.5	5	13019	1.7	3
	0	561	3.5		4349	1.5	
4	10	522751	1.4	797	1166977	0.7	303
	1	12629	8.9	19	128547	22.6	33
	0.1	1436	3.9	2	13771	1.1	4
	0	656	2.4		3849	21.1	
5	10	405311	2.9	615	1895001	0.0	418
	1	7301	23.0	11	238293	17.2	53
	0.1	1118	28.9	2	34562	22.0	8
	0	659	4.2		4535	35.9	
6	10	393392	7.0	593	2167059	4.5	439
	1	9624	3.6	15	334969	41.9	68
	0.1	1287	14.5	2	32062	12.6	7
	0	663	14.1		4932	33.1	
7	10	1424511	1.2	1279	2124322	1.7	479
	1	609032	0.8	547	1109105	2.5	250
	0.1	40304	1.6	36	74463	27.1	17
	0	1113	12.1		4432	21.2	
8	10	1411858	5.0	1434	2577122	0.9	416
	1	39122	11.6	40	679399	18.3	110
	0.1	3473	1.7	4	104699	23.5	17
	0	984	2.8		6197	20.1	
9	10	2753898	4.8	1223	2876882	4.5	429
	1	1052038	14.2	467	1141493	15.8	170
	0.1	61041	9.1	27	246653	47.2	37
	0	2252	51.2		6710	31.4	

2.2 Competitive Assay Screen (MTP)

The 9 antibodies were coated on an MTP at 10 ug/mL by passive absorption, and tested for response in a competitive assay with PCP calibrators in Low BSA buffer, with the 2 AP conjugates at 1:10,000 from the stock solution.

Several of the antibodies showed modulation in the competitive assay with either AP conjugate #4 or 5. Antibodies # 2, 3, 5 and 6 were chosen to move on to the Theranos System with the Theranos in-house AP conjugate #5.

Figure [SEQ Figure * ARABIC]: Competitive Assay Screen (MTP)

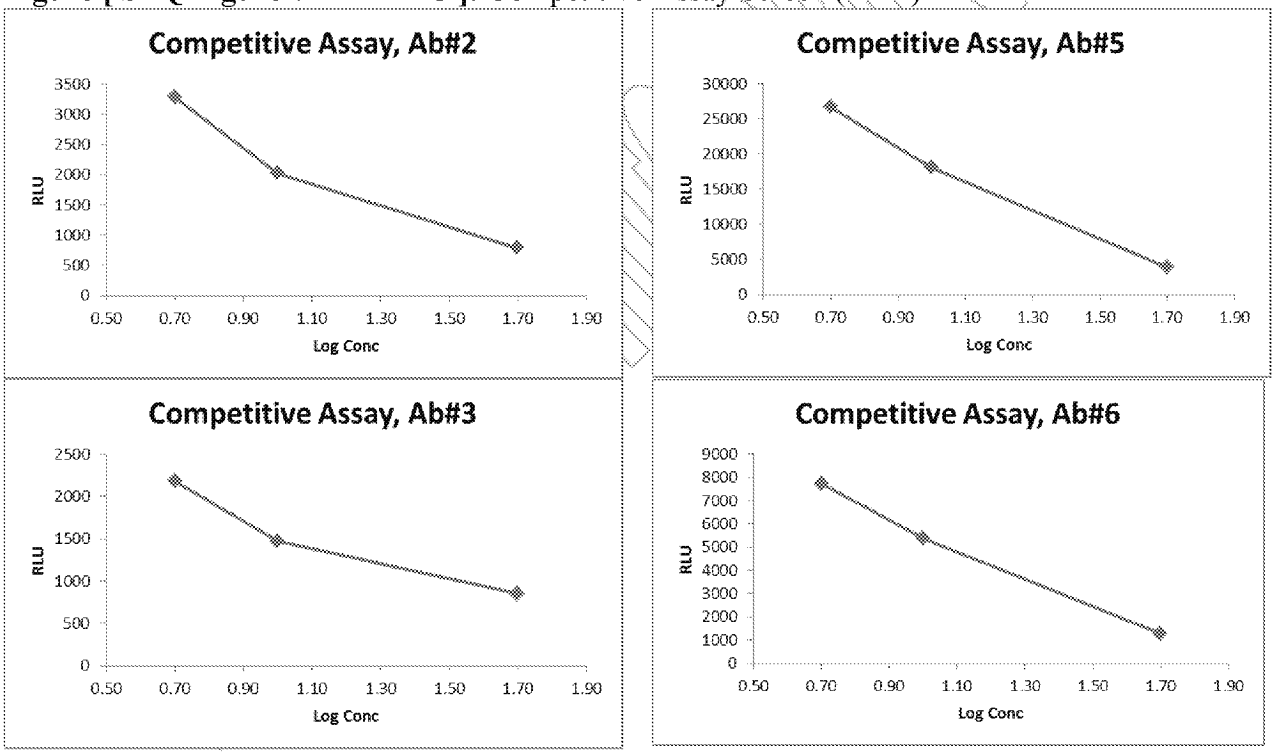


Table [SEQ Table * ARABIC]: Competitive Assay Screen (MTP)

Conjugate #:		4			5		
Ab#	**[PCP] ng/mL**	**Mean RLU**	**CV %**	**Mod**	**Mean RLU**	**CV %**	**Mod**
1	50	3296	10.0	42	49459	2.9	10
	10	13627	10.4	10	148510	0.9	3
	5	17674	5.2	8	215547	2.6	2
	0	139558	1.5		519006	1.1	
2	50	307	7.4	10	786	4.9	29
	10	458	5.0	7	2021	31.5	11
	5	584	2.2	5	3289	10.0	7
	0	2983	8.1		23059	7.4	
3	50	361	10.6	8	852	9.5	28
	10	428	11.9	6	1471	0.7	16
	5	493	5.2	6	2185	4.5	11
	0	2720	9.6		23962	10.4	
4	50	473	4.8	5	4962	2.3	6
	10	841	5.4	3	13532	2.6	2
	5	1144	6.2	2	18712	0.5	2
	0	2528	5.4		29428	2.0	
5	50	374	8.2	3	3943	2.8	11
	10	590	6.9	2	18073	3.1	2
	5	689	4.1	1	26727	3.6	2
	0	948	0.3		42409	12.6	
6	50	302	0.0	2	1262	5.6	11
	10	401	8.2	1	5376	2.2	3
	5	494	10.8	1	7739	12.8	2
	0	498	0.5		14174	1.6	
7	50	57000	6.2	2	301293	3.7	2
	10	84290	1.4	1	450316	0.8	1
	5	89917	1.5	1	497948	0.4	1
	0	125708	3.3		545138	2.9	
8	50	1972	80.8	1	60644	7.5	3
	10	2127	32.6	1	136047	8.7	1
	5	2147	0.5	1	163606	9.0	1
	0	2713	8.5		186031	10.5	
9	50	89750	11.1	4	361779	5.3	2
	10	233633	2.9	1	625310	11.1	1
	5	252677	1.8	1	724516	0.8	1
	0	326448	2.4		703535	3.7	

2.3 Theranos System 3.0 Screen

The 4 best-modulating antibodies were tested on the Theranos System 3.0 with secondary antibody as the capture surface, unlabeled anti-PCP Ab mixed into the sample along with the Theranos PCP-AP conjugate at the same dilution used on the MTP of 1:10,000 (further diluted 1:10 in the Theranos System), and a 1:10 sample dilution. The response on the Theranos System was good for Ab #2 and 3, showing sensitivity at the 25 ng/mL level needed for determination of positive results in urine. Antibodies # 5 and 6 did not show good response in the competitive assay on the Theranos System despite showing good response on the MTP. Differences between the MTP assay and the Theranos System assay include temperature, coating method, and coating surface.

Since the signal was very high overall, the best sheep anti-PCP antibody and the best mouse Anti-PCP antibody were re-tested with a 1:1,000,000 dilution of the PCP-AP conjugate (further diluted 1:10 in the Theranos System). With this concentration of PCP-AP, the mouse Ab #5 showed only 3-fold modulation, while the sheep Ab #3 showed excellent modulation in the competitive assay. Antibody #3 was chosen as the final candidate.

Table [SEQ Table * ARABIC]: Theranos System 3.0 Screen, PCP-AP 1:10,000

Ab #	[PCP] ng/mL	Mean RLU	CV %	Modulation
2	500	432006	12.5	8
	25	1374164	4.2	2
	0	3288517	3.8	
3	500	287261	14.9	9
	25	728930	19.4	4
	0	2622603	3.2	
5	500	3144635	7.9	1
	25	4407380	6.9	1
	0	3966447	13.1	
6	500	3443037	5.8	1
	25	3929803	8.8	1
	0	4158831	8.4	

Table [SEQ Table * ARABIC]: Theranos System 3.0 Screen, PCP-AP 1:1,000,000

Ab #	[PCP] ng/mL	Mean RLU	CV %	Modulation
3	500	3187	16.7	66
	25	13532	2.3	16
	0	210378	13.8	
5	500	110689	9.9	3
	25	309853	0.2	1
	0	306460	5.0	

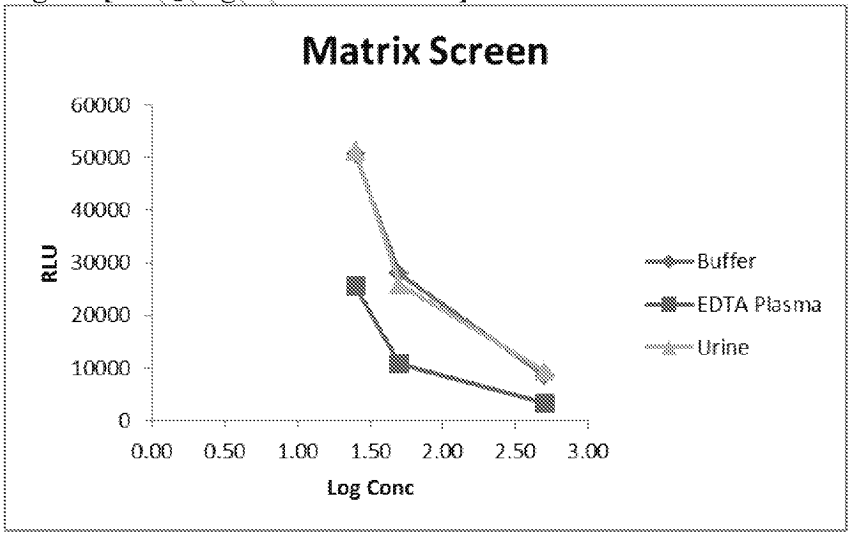
2.4 Matrix Screen

Since the Theranos PCP assay is intended to measure multiple matrixes including serum, plasma and urine, a matrix screen was performed to determine the response in the appropriate matrixes. This screen was done with the starting sample dilution of 1:10. Urine and buffer matrixes showed very similar dose response, and although plasma showed a lower overall signal, the modulation was very good and comparable to the modulation in buffer or urine. Therefore buffer calibrators were used for assay optimization, and matrix tests were repeated after the assay was optimized and the sample dilution increased.

Table [SEQ Table * ARABIC]: Matrix Screen

Condition	[PCP] ng/mL	Mean RLU	CV %	Mod
Low BSA Buffer	500.00	8529	2.6	23
	50.00	27931	24.1	7
	25.00	50709	8.3	4
	10.00	72010	7.8	
	5.00	125696	12.5	
	0.00	200353	19.4	
EDTA Plasma	500.00	3302	5.1	29
	50.00	10749	21.7	9
	25.00	25480	15.4	4
	0.00	94472	10.5	
Urine	500.00	9430	12.8	22
	50.00	25896	6.2	8
	25.00	51190	1.5	4
	0.00	203526	5.6	

Figure [SEQ Figure * ARABIC]: Matrix Screen



2.5 Alkaline Phosphatase Conjugate Stabilizers

To determine the optimal stabilizing buffer for the PCP-AP conjugate, 2 commercial buffers and one in-house formulation was tested. The Theranos Small Molecule AP Conjugate Stabilizer consists of 0.1 mM Zn^{2+}, 5 mM Mg^{2+} and 0.03% BSA with 0.05 % Sodium Azide in 50mM TBS pH 8.0. All of the stabilizers performed well in the assay but the Theranos in-house formulation showed the best modulation and was chosen as the final assay condition.

Table [SEQ Table * ARABIC]: Alkaline Phosphatase Conjugate Stabilizers

AP Stabilizing Buffer	[PCP] ng/mL	Mean RLU	CV %	Modulation
Theranos SM AP Stabilizer	500.00	4175	25.5	25
	25.00	24468	12.1	4
	0.00	104418	14.7	
Stabilzyme AP	500.00	4587	26.4	22
	25.00	23229	14.8	4
	0.00	101655	19.5	
Biostab	500.00	4988	7.9	22
	25.00	25206	14.1	4
	0.00	107958	8.4	

2.6 Sample Dilution

To reduce matrix effects, higher sample dilutions were tested. A dilution of 1:50 showed more than adequate sensitivity while reducing potential matrix effects in the assay. The 1:50 sample dilution was chosen for further assay optimization. A 1:100 sample dilution protocol was later made available to test.

Table [SEQ Table * ARABIC]: Sample Dilution

Sample Dilution	[PCP] ng/mL	Mean RLU	CV %	Mod
1:10	500	3615	10.5	29
	50	12211	21.3	9
	25	19087	6.8	5
	0	104418	14.7	
1:25	500	2810	5.3	50
	50	9336	16.9	15
	25	15643	12.5	9
	0	140554	14.8	
1:50	500	3819	14.2	38
	50	13648	4.7	11
	25	24709	4.7	6
	0	145829	11.9	

2.7 Reagent Incubation Time

Sample mixture and substrate incubation times of 10-10, 5-5 and 2-1 minutes were tested. All of the incubation times performed well, however the 5-5 minute incubation time showed the best overall response and shortened the assay reagent time to a total of 10 minutes. The assay would also perform well with 10-10 or 2-1 minute incubation times. The CV % is not shown because when using 1 minute substrate incubation time, the duplicate tips on the cartridges must be considered as 2 separate calibrations due to the time difference in MPT read and the rate of the reaction.

Table [SEQ Table * ARABIC]: Reagent Incubation Time

Incubation Time (Min)	[PCP] ng/mL	Mean RLU	Mod
10-10	500	3819	38
	50	13648	11
	25	24709	6
	0	145829	
5-5	500	1297	46
	50	4802	12
	25	8410	7
	0	59351	
2-1	500	312	30
	50	1215	8
	25	1600	6
	0	9494	

2.8 Reagent Titration

To determine the optimal concentration of antibody and PCP-AP conjugate, the reagents were titrated. The best response was shown with a loading concentration of 5 ug/mL CAb and 1:1,000,000 PCP-AP conjugate. Both reagents are spiked in at a 1:10 final dilution during the sample preparation by the onboard protocol.

Table [SEQ Table * ARABIC]: Reagent Titration

Loading [Ab] ug/mL	Final [Ab] ug/mL	Loading PCP-AP, Dilution from stock	Final PCP-AP, Dilution from Stock	[PCP] ng/mL	Mean RLU	CV %	Mod
10	1	1:1M	1:10M	500	1297	10.8	46
				50	4802	4.2	12
				25	8410	13.2	7
				0	59351	14.0	
25	5	1:5M	1:50M	500	621	7.8	20
				50	2169	17.0	6
				25	3423	5.2	4
				0	12665	6.1	
5	0.5	1:1M	1:10M	500	1090	12.5	57
				50	4310	23.8	14
				25	6614	17.2	9
				0	62225	8.1	
5	0.5	1:500K	1:5M	500	2365	11.2	40
				50	8426	32.1	11
				25	13629	12.3	7
				0	95124	6.8	

2.9 Standard Curve in Buffer and Serum

With the 1:50 sample dilution, there was a matrix effect between buffer and serum, while urine can be calculated on the buffer curve. Therefore both standard curves were generated and used to calculate results in the appropriate matrixes.

Table [SEQ Table * ARABIC]: Standard Curve in Serum

[PCP] ng/mL	Signal, RLU		Conc, ng/mL		
	Mean RLU	CV %	Mean Conc	CV %	% Recovery
500	675	8.4	503	13.3	101
50	2707	2.6	53	4.3	105
25	5046	8.0	18	14.2	72
10	6348	14.5	12	27.9	122
5	9940	19.8	5	41.8	105
0	36993	12.5	0	35.5	

Table [SEQ Table * ARABIC]: Standard Curve in Buffer

[PCP] ng/mL	Signal, RLU		Conc, ng/mL		
	Mean RLU	CV %	Mean Conc	CV %	% Recovery
500	912	20.7	674	71.8	135
50	3543	19.7	51	20.8	102
25	5987	9.4	28	12.0	111
10	11246	8.6	9	19.8	93
5	13823	13.0	6	35.1	114
0	48754	17.3	0	113.0	

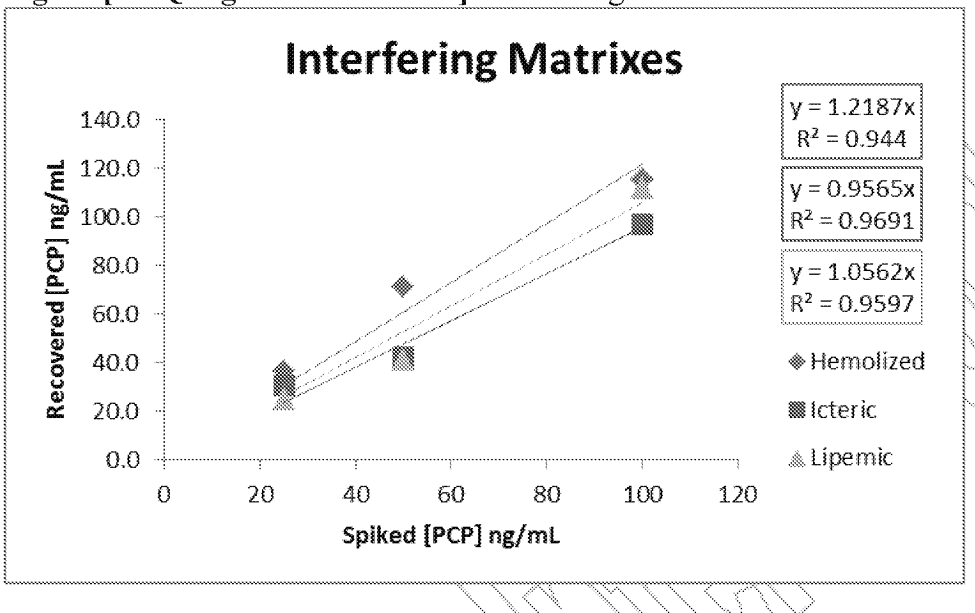
2.10 Interfering Matrixes

The effect of potentially interfering matrixes was tested by measuring spike recovery in grossly hemolyzed, icteric and lipemic serum. Recovery – calculated on a serum standard curve – was excellent in icteric and lipemic sera, but slightly high in hemolzyed serum. Therefore, grossly hemolyzed samples may show falsely elevated results. This should not create false positives in the assay, since the un-spiked negative sample was still well below the cutoff level of 25 ng/mL.

Table [SEQ Table * ARABIC]: Interfering Matrixes

Matrix	Spiked [PCP] ng/mL	Signal, RLU		Conc, ng/mL		
		Mean RLU	CV %	Mean Conc	CV %	% Recovery
Hemolized	100	1730	19.4	115	29.0	115
	50	2264	6.9	71	11.3	142
	25	3452	14.3	36	26.8	145
	0	32889	2.9	OORL		
Icteric	100	1874	6.7	97	11.5	97
	50	3253	23.4	42	41.4	85
	25	3739	9.1	31	14.7	123
	0	34048	5.6	OORL		
Lipemic	100	1808	24.7	112	38.5	112
	50	3137	7.5	41	12.2	83
	25	4278	15.9	25	26.9	100
	0	38047	8.7	OORL		

Figure [SEQ Figure * ARABIC]: Interfering Matrixes



2.11 Normal Sample Screen

A set of 10 normal urine and 10 normal EDTA plasma samples were screened. Urine samples results were calculated on the low BSA buffer curve and the plasma samples were calculated on the serum standard curve. The Bioquant ELISA was run as a semi-quantitative assay by creating a standard curve with the cutoff calibrator. All samples were negative in the Bioquant ELISA, and in the Theranos assay – the urine samples were also negative on the DRG Quickscreen test (510k for IVD).

Positive Cutoff for PCP
Urine = 25 ng/mL
Serum/Plasma = 15 ng/mL (10 ng/mL for confirmation)

Table [SEQ Table * ARABIC]: Normal Plasma Screen

Sample ID	Bioquant Result, ng/mL	Theranos Result		
		Signal, RLU		Conc, ng/mL
		Mean RLU	CV %	
E1	OORL	48416	5.4	OORL
E2	OORL	40207	9.2	OORL
E3	OORL	38715	9.4	OORL
E4	OORL	31358	24.0	OORL
E5	OORL	38041	12.1	OORL
E6	OORL	50698	13.2	OORL
E7	OORL	55684	6.3	OORL
E8	OORL	47084	13.0	OORL
E9	OORL	40316	12.9	OORL
E10	OORL	54252	14.1	OORL

Table [SEQ Table * ARABIC]: Normal Urine Screen

Sample ID	Bioquant Result, ng/mL	DRG Result	Theranos Result		
			Signal, RLU		Conc, ng/mL
			Mean RLU	CV %	
U1	0.4	Neg	43534	12.5	OORL
U2	1.0	Neg	41125	8.0	OORL
U3	1.0	Neg	44541	23.6	OORL
U4	1.3	Neg	43431	13.3	OORL
U5	0.2	Neg	38247	10.0	OORL
U6	OORL	Neg	48974	25.8	OORL
U7	0.5	Neg	48696	0.3	OORL
U8	0.3	Neg	53489	13.3	OORL
U9	1.6	Neg	39294	9.8	OORL
U10	1.2	Neg	28224	15.6	OORL

2.12 Clinical Samples

Since positive clinical samples could not be obtained, the 10 normal plasma and urine samples previously screened were spiked with different levels of PCP, including one sample spiked at the appropriate cutoff for urine and serum. The samples were measured in the Theranos System, the Bioquant ELISA, and the urine samples were tested in the DRG Quickscreen strips (510k for IVD).

Positive results greater than or equal to the cutoff are highlighted in pink and negative results are highlighted in green. The Theranos result agreed with the Bioquant and DRG result in all cases.

Positive Cutoff for PCP:
Urine = 25 ng/mL
Serum/Plasma = 15 ng/mL (10 ng/mL for confirmation)

Table [SEQ Table * ARABIC]: Spiked Plasma Clinical Correlation

Sample ID	Spiked [PCP] ng/mL	Bioquant, ng/mL	Theranos, ng/mL
E1-S	90	95	88
E2-S	85	73	68
E3-S	80	88	66
E4-S	75	50	75
E5-S	70	53	68
E6-S	55	52	43
E7-S	50	34	38
E8-S	35	31	31
E9-S	30	28	26
E10-S	15	23	15

Table [SEQ Table * ARABIC]: Spiked Urine Clinical Correlation

Sample ID	Spiked [PCP] ng/mL	Bioquant, ng/mL	DRG Result	Theranos, ng/mL
U1-S	90	72	Pos	75
U2-S	85	60	Pos	69
U3-S	80	51	Pos	55
U4-S	75	50	Pos	48
U5-S	70	47	Pos	44
U6-S	65	44	Pos	39
U7-S	50	37	Pos	39
U8-S	45	34	Pos	37
U9-S	30	23	Pos	29
U10-S	25	19	Neg	20

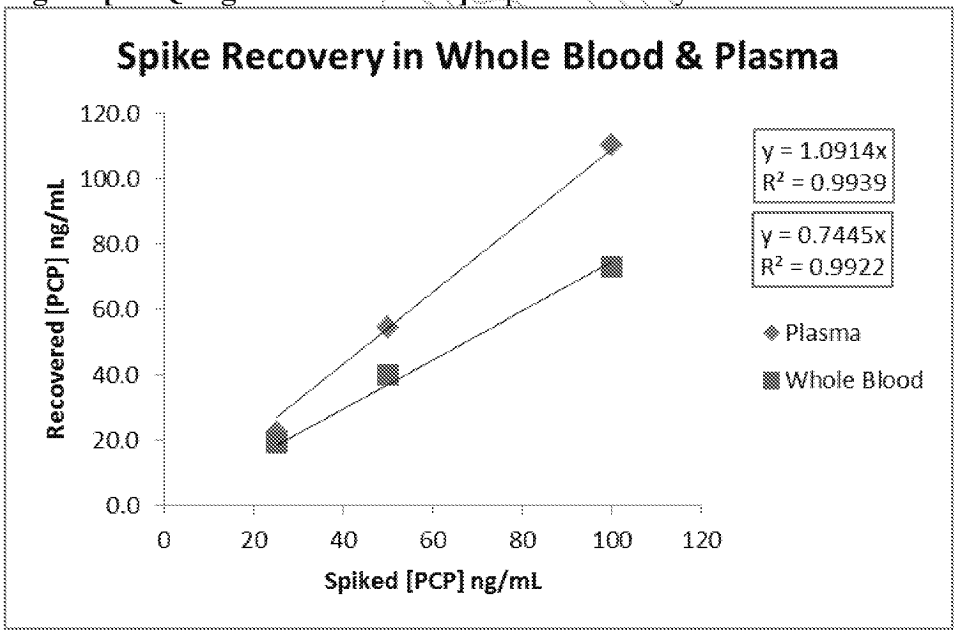
2.13 Whole Blood and Plasma Spike Recovery

Spike recovery was tested in whole blood and EDTA plasma at 3 levels. Spike recovery was excellent in plasma and approximately 75% in whole blood.

Table [SEQ Table * ARABIC]: Spike Recovery in Whole Blood and Plasma

Matrix	Spiked [PCP] ng/mL	Recovered [PCP] ng/mL	CV %	% Recovery
Whole Blood	100	72.8	12.0	73
	50	40.1	9.9	80
	25	19.3	14.6	77
	0	OORL	-	-
Plasma (EDTA)	100	110.3	13.8	110
	50	54.7	13.0	109
	25	22.5	21.0	90
	0	OORL	-	-

Figure [SEQ Figure * ARABIC]: Spike Recovery in Whole Blood and Plasma



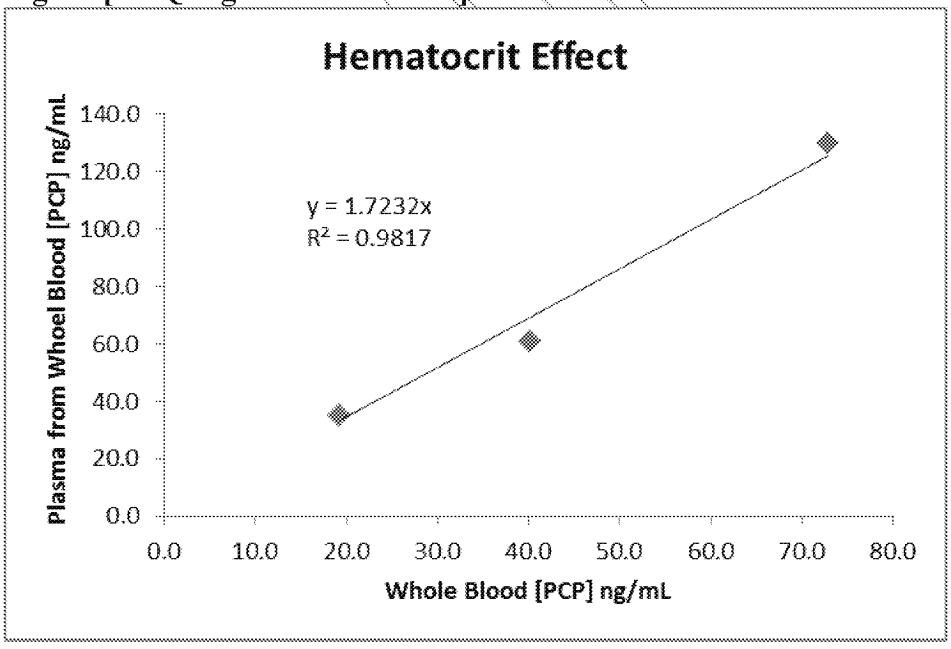
2.14 Hematocrit Effect

The hematocrit effect was tested by measuring spiked whole blood samples on the Theranos System, then measuring the plasma prepared by centrfuging the spiked whole blood. The results in plasma were approximately 1.7 times higher than the whole blood, as expected based on average blood hematocrit. If whole blood results are compared to plasma or serum, the results can be adjusted based on the sample hematocrit.

Table [SEQ Table * ARABIC]: Hematocrit Effect

Whole Blood		Plasma from Whole Blood	
Mean Conc, ng/mL	**CV %**	**Mean Conc, ng/mL**	**CV %**
72.8	12.0	129.6	12.3
40.1	9.9	60.9	14.5
19.3	14.6	34.9	16.1
OORL		OORL	

Figure [SEQ Figure * ARABIC]: Hematocrit Effect



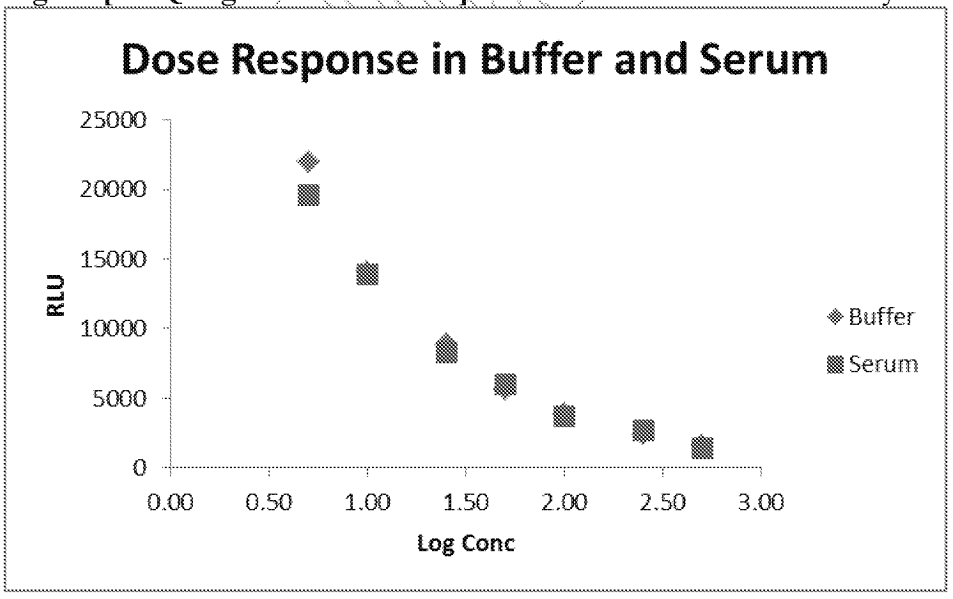
2.15 Matrix Effects with Final Assay Conditions

A 100x sample dilution protocol was created and a standard curve in serum was produced and compared to the buffer standard curve. With the increased sample dilution, the desired effect of eliminating the matrix effect was achieved - the serum results were accurately and precisely calculated based on the buffer standard curve. Under final assay conditions, only one calibration is necessary for urine, serum or plasma matrixes.

Table [SEQ Table * ARABIC]: Standard Curve in Serum

[PCP] ng/mL	Signal, RLU		Conc, ng/mL		
	Mean RLU	CV %	Mean Conc	CV %	% Recovery
500	1442	15.2	476.2	16.7	95
250	2659	31.3	218.4	36.6	87
100	3718	10.6	106.8	18.5	107
50	6030	4.9	46.5	7.7	93
25	8276	12.6	27.5	22.9	110
10	13900	13.5	11.5	24.6	115
5	19589	8.1	5.9	15.8	118
0	53505	16.2	OORL	-	-

Figure [SEQ Figure * ARABIC]: Matrix Effects with Final Assay Conditions



2.16 Determination of LLOQ and ULOQ

The buffer standard curve was used to determine the LLOQ and ULOQ of the assay in all matrixes. Theranos calibration software was used to fit the data and determine the LLOQ and ULOQ in urine according to FDA guidelines for calibrating ELISA assays. The LLOQ was 5 ng/mL and the ULOQ was 500 ng/mL.

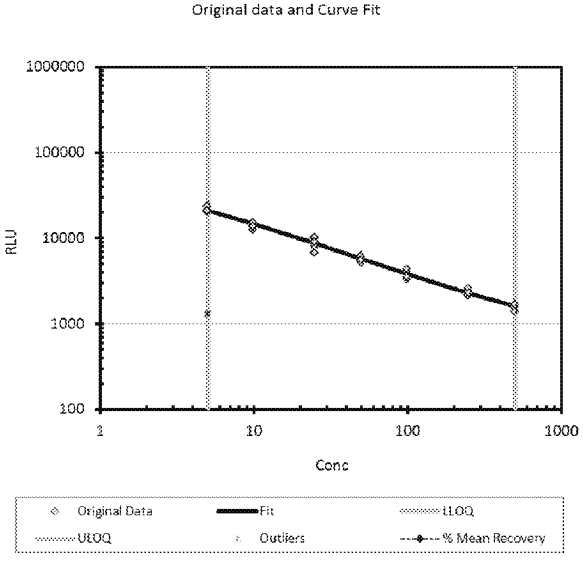
Table [SEQ Table * ARABIC]: Determination of LLOQ and ULOQ

[PCP] ng/mL	Signal, RLU		Conc, ng/mL		
	Mean RLU	CV %	Mean Conc	CV %	% Recovery
500	1543	9.4	479.7	8.3	96
250	2379	6.9	233.0	13.0	93
100	3882	12.2	99.9	20.7	100
50	5623	7.0	52.5	11.1	105
25	8817	16.6	25.9	30.2	104
10	14064	7.6	11.0	13.8	110
5	22016	7.7	5.1	5.6	103
0	65201	6.8	OORL	-	-

Conc = 1.624 * (((67365.805 - 551.410) / (S - 551.410)) - 1) ^ (1 / 0.721)
SMin = 1509, SMax = 23473

Table [SEQ Table * ARABIC]: Calibration Parameters

Parameter	Value	Unit
LLOQ	5.00	ng/mL
ULOQ	500.00	ng/mL
LLOQ accuracy	96	%
LLOQ precision	17.0	%
ULOQ accuracy	117	%
ULOQ precision	20.3	%
Average Residuals	8	%
Error in prediction: Best case	14	%
Error in prediction: Expected	15	%



2.17 Calibration Verification

To verify the Theranos System calibration, drugs of abuse (DOA) urine controls were obtained from Synergent Biochem and tested in the Theranos System. The Theranos results were within the allowable range and matched very closely with the GC-MS reported result. The Theranos result also matched with the DRG Quickscreen result for all levels. The Bioquant kit over-recovered all levels, including 570-1 which is reported to be below the urine cutoff of 25 ng/mL and showed up negative in the DRG Quickscreen (510k for IVD) and the Theranos System.

Positive results greater than or equal to the cutoff are highlighted in pink and negative results are highlighted in green.

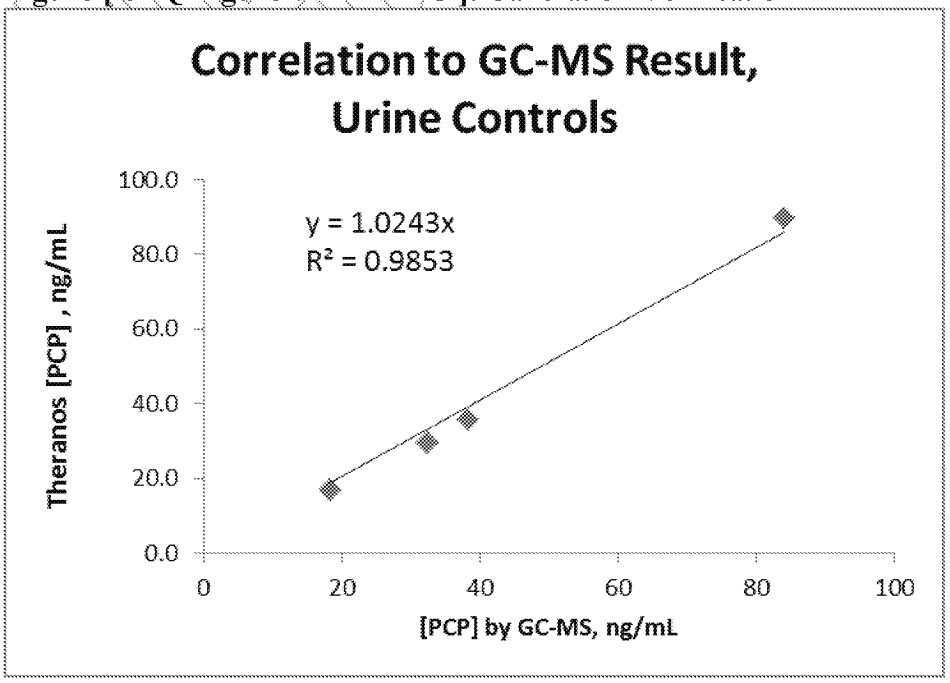
Positive Cutoff for PCP in urine = 25 ng/mL

Table [SEQ Table * ARABIC]: Calibration Verification

Level	Range, ng/mL	GC-MS, ng/mL	Bioquant, ng/mL	DRG Result*	Theranos, ng/mL	Theranos % Recovery to GC-MS
571-1	0	0	OORL	Neg	OORL	
570-1	14.6-22.0	18.3	31.5	Neg	16.7	91
570-2	26.0 - 39.0	32.5	46.1	Pos	29.6	91
571-2	30.7 - 46.1	38.4	57.9	Pos	35.5	92
571-3	67.2 - 100.8	84.0	117.3	Pos	89.7	107

* DRG test is 510k for IVD

Figure [SEQ Figure * ARABIC]: Calibration Verification



2.18 Cross Reactivity and Interference

Although some cross reactants were not commercially available to test, the DOA urine controls from Synergent Biochem have reported values by GC-MS for a panel of DOA substances. All of these substances were present in excess of up to 40 times the PCP concentration. Recovery for all 5 control levels corresponded with the reported PCP concentration as measured by GC-MS in the presence of an excess of multiple non-target drugs, therefore no cross reactivity or interference from these substances was observed.

Table [SEQ Table * ARABIC]: Cross Reactivity and Interference

	Control Level:	**571-1**	**570-1**	**570-2**	**571-2**	**571-3**
Non-Target Drug Levels by GC-MS, ng/mL	11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid	0	40.4	104.5	113	386
	Amitriptyline	0	831	1470	1910	3510
	Benzoylecgnine	0	229	362	428	776
	d-Amphetamine	0	714	1086	1350	2827
	Methadone	0	207	394	418	897
	Methaqualone	0	238	368	428	945
	Morphine	0	268	406	480	951
	Oxazepam	0	142	266	306	611
	Propoxyphene	0	230	358	464	938
	Secobarbital	0	223	356	468	892
	[PCP] ng/mL GC-MS	0	18.3	32.5	38.4	84.0
	[PCP] Min, ng/mL	0	14.6	26.0	30.7	67.2
	[PCP] Max, ng/mL	0	22.0	39.0	46.1	100.8
	Theranos [PCP] Result, ng/mL	OORL	16.7	29.6	35.5	89.7

2.19 Assay Formats for Multiplexing

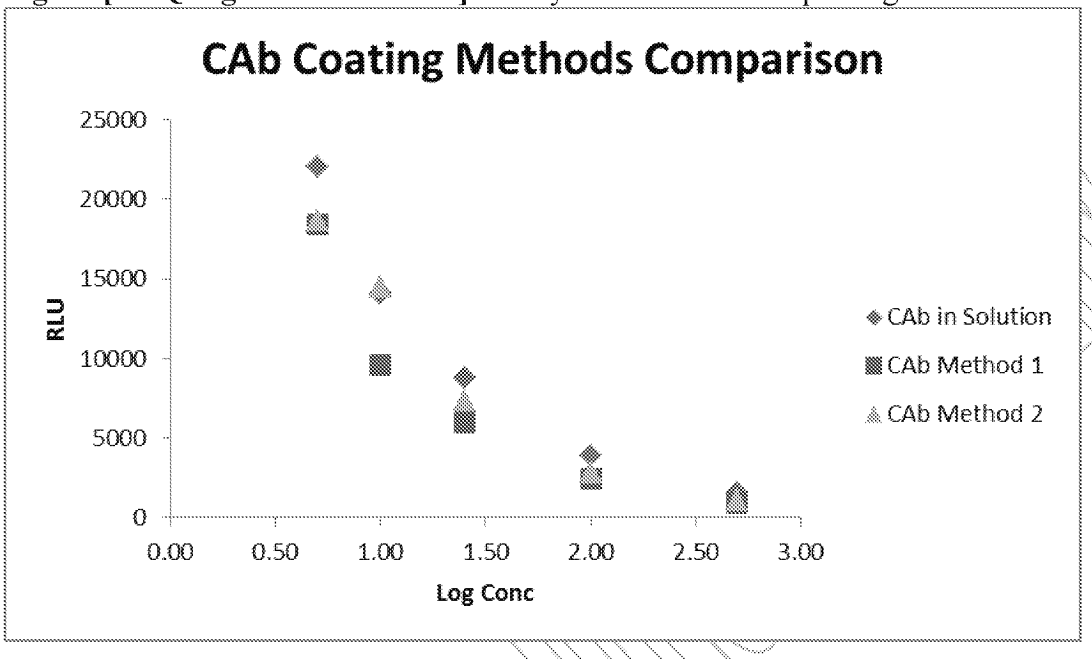
In order to simplify the assay format for easier multiplexing in the drugs screening panels, 2 methods were tested for coating the anti-sheep serum on the surface rather than mixing it in solution in a homogenous competitive assay. Both methods showed very comparable results to the capture antibody in solution. Method 1 was chosen as the final assay condition.

- CAb in solution: 500 ng/mL Sheep Anti-PCP PAb in solution with sample mixture with 20 ug/mL UltraAvidin, 10 ug/mL Biotinylated Anti-Sheep Ab coated on the surface
- Method 1: 20 ug/mL Anti-Sheep Ab, 500 ng/mL Sheep Anti-PCP PAb
- Method 2: 20 ug/mL UltraAvidin, 10 ug/mL Biotinylated Anti-Sheep Ab, 500 ng/mL Sheep Anti-PCP PAb

Table [SEQ Table * ARABIC]: Assay Formats for Multiplexing

Condition	[PCP] ng/mL	Signal, RLU			Conc, ng/mL		
		Mean RLU	CV %	Mod	Mean	CV %	% Recovery
CAb in Solution	500	1543	9.4	42	508	15.8	102
	100	3882	12.2	17	105	21.4	105
	25	8817	16.6	7	25	31.7	102
	10	14064	7.6	5	11	13.2	107
	5	22016	7.7	3	5	12.4	100
	0	65201	6.8		OORL	-	-
CAb Method 1	500	914	18.4	40	511	23.4	102
	100	2480	13.9	15	106	20.7	106
	25	5944	5.2	6	23	8.8	92
	10	9600	8.5	4	11	12.0	109
	5	18422	10.7	2	5	11.1	99
	0	36176	21.4		OORL	-	-
CAb Method 2	500	1046	28.6	35	681	74.6	136
	100	2810	6.4	13	98	8.9	98
	25	7328	2.1	5	27	2.9	108
	10	14474	4.1	3	9	8.1	90
	5	18632	12.2	2	6	26.4	111
	0	36375	14.3		OORL	-	-

Figure [SEQ Figure * ARABIC]: Assay Formats for Multiplexing



2.20 Stability

Stability studies are ongoing.

3 REFERENCES

- Draft Guidance for Industry and FDA Staff: Premarket Submission and Labeling Recommendations for Drugs of Abuse Screening Tests. Issued December 2, 2003.

- Bassan DM, Erdmann F, Krüll R. "Quantitative determination of 43 common drugs and drugs of abuse in human serum by HPLC-MS/MS." Anal Bioanal Chem. 2011 Apr;400(1):43-50. Epub 2011 Feb 18.

- Chimalakonda KC, Hailey C, Black R, Beekman A, Carlisle R, Lowman-Smith E, Singletary H, Owens SM, Hendrickson H. "Development and validation of an LC-MS/MS method for determination of phencyclidine in human serum and its application to human drug abuse cases." Anal Methods. 2010 Sep 1;2(9):1249-1254.

- Tai SS, Prendergast JL, Sniegoski LT, Welch MJ, Phinney KW, Zhang NF. "Certification of drugs of abuse in a human serum standard reference material: SRM 1959." Anal Bioanal Chem. 2010 May;397(2):501-9. Epub 2010 Mar 24.

- Ferguson AM, Garg U. "Quantitation of phencyclidine (PCP) in urine and blood using gas chromatography-mass spectrometry (GC-MS)." Methods Mol Biol. 2010;603:461-7.

- Hull MJ, Griggs D, Knoepp SM, Smogorzewska A, Nixon A, Flood JG. "Postmortem urine immunoassay showing false-positive phencyclidine reactivity in a case of fatal tramadol overdose." Am J Forensic Med Pathol. 2006 Dec;27(4):359-62.

- Wohlfarth A, Weinmann W, Dresen S. "LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum." Anal Bioanal Chem. 2010 Apr;396(7):2403-14. Epub 2010 Jan 13.