DHEAS Assay Development Report

Theranos, Inc.

June, 2013

Prepared by: Ran Hu

TABLE OF CONTENTS

[TOC \O "1-3" \H \Z \U]LIST OF TABLES

[TOC \h \z \c "Table"]

LIST OF FIGURES

[TOC \h \z \c "Figure"]

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

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

Dehydroepiandrosterone sulfate (DHEAS) and Dehydroepiandrosterone (DHEA) are steroid hormones produced by the adrenals. DHEAS is the sulfate derivative of DHEA. It is the most abundant hormone in human body and a precursor of all sex steroids. DHEA-S is also produced in the gonads, adipose tissue and the brain. In female, it is produced in ovaries as well.

DHEA(S) level in blood declines after birth until about the age of five to seven, and then it starts to increase to reach the top level at age of 20 to 30. The normal level of DHEAS is up to 300ug/dL in female adults and 500ug/dL in male adults. Lowered levels of DHEAS have been reported in hypoadrenalism. Elevated levels have been seen in polycystic ovarian syndrome (PCOS), female hirsutism, and individuals having prolonged physical stress.

DHEAS has a slow metabolic rate so it maintains high blood level which is about thousand fold higher than DHEA. It does not circulate bound to sex hormone-binding globulin. DHEAS is an important biomarker for evaluating the function of adrenals. Measurement of DHEAS has been used in diagnose of tumors in the cortex of the adrenal gland (adrenocortical tumors) and of adrenal cancers. It is also helpful in the investigations of abnormal hair growth (hirsutism) and balding (alopecia) in women, in the assessment of adrenarche and delayed puberty, in the evaluation of patients with polycystic ovarian syndrome (PCOS) and etc.

1.2 Assay specifications

Theranos DHEAS assay is an immunoassay utilizing an anti-DHEAS antibody to quantitatively measure DHEAS concentration in whole blood, EDTA plasma, heparin plasma and serum. The quantification range is from 41ng/ml to 10000ng/ml (4ug/dL to 1000ug/dL) in the above mentioned sample types.

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

The following assay was used as reference method:

DHEAS, SIEMENS Immulite 2000, assay quantification range 150ng/ml to 10000ng/ml (LLOQ 150ng/ml)

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

A competitive immunoassay using anti-DHEAS antibody was developed for the quantitative determination of DHEAS in serum, plasma and whole blood.

In this assay, a rabbit anti-DHEAS polyclonal antibody is used as capture agent. Reaction tips are coated with Ultra-avidin first, and followed by coating of secondary antibody biotin labeled goat anti-rabbit IgG. The primary capture antibody rabbit anti-DHEAS polyclonal antibody is prepared in solution. Serum, plasma or whole blood samples are diluted 50 folds with sample diluent and mixed with anti-DHEAS antibody and DHEAS-alkaline phosphatase conjugate. The mixture is then incubated with coated tips. DHEAS in sample and DHEAS-AP conjugate competitively bind to anti-DHEA antibody which binds to coated tips. After incubation, the tips are washed with wash buffer and incubated with AP substrate. The chemiluminescence results are measured and reported as Relative Light Units (RLU). A calibration curve is generated by plotting the measured response (RLU) vs. concentration of each calibrator. DHEAS concentration of unknown sample is calculated from calibration curve.

Table [SEQ Table * ARABIC]: DHEAS assay materials in final assay procedure

Name	Supplier	Catalog number
Dehydroepiandrosterone Sulfate	Cerilliant	D-065
Biotin labeled Goat anti-Rabbit IgG	Southern Biotech	4041-08
Rabbit anti-DHEAS polyclonal antibody	Lifespan Biosciences	LS-C85783
Tris buffer (powder)	Sigma	T6664
Bovine serum albumin	Sigma	A3059
Sucrose	Sigma	S5016
5% Sodium Azide solution	VWR	101320-516
Carbonate-bicarbonate buffer	Sigma	C3041
StabilZyme AP stabilizer	SurModics	SA01
Wash buffer	In house	
UltraAvidin	Leinco	A110
AP substrate buffer	In house	Current Lot 19122012 NGB-ALP
DHEAS-AP conjugate	In house	Current Lot DHEAS-EMCH-AP_01_010813

1.5 Raw data storage

Raw data of assay development were stored in Elog #837 and Theranos notebook #477.

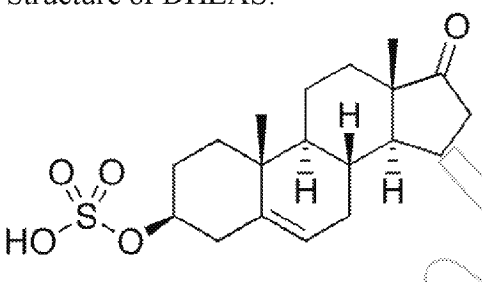
2 ASSAY DEVELOPMENT[TC "ASSAY OPTIMIZATION" \F C \L "2"]

2.1 Initial antibody screening on MTP

2.1.1 Antibody screen with DHEAS-AP conjugate direct binding

During initial assay development stage, thirteen anti-DHEAS antibodies were screened on multi-titer plate (MTP) with antibody coating and conjugate direct binding format. Two DHEAS-AP conjugate with different linkers were obtained from Theranos in-house chemistry group. Because the analyte DHEAS is a small molecule (M.W. 368.5Da), the assay is targeted to be a competitive immunoassay format.

Structure of DHEAS:



Methods:

Because the available anti-DHEAS antibodies included mouse monoclonal antibodies, rabbit polyclonal antibody and sheep polyclonal antibody, "2-stack coating" format with secondary antibodies as first layer on plate were used to minimize the differences among anti-DHEAS antibodies from different sources.

The MTP was coated with goat anti-mouse IgG (for antibody #1 to #11), or goat anti-rabbit IgG (for antibody #12), or goat anti-sheep IgG (for antibody #13) at 20ug/ml in coating buffer. Anti-DHEAS antibodies #1 to #11 were prepared at 10, 1, and 0.1ug/ml in blocking buffer, #12 and #13 were prepared at 1:1000, 1:10000, and 1:100000 dilutions in blocking buffer. Anti-DHEAS antibodies were incubated with secondary antibody coated plate first. Then DHEAS-AP conjugate #1 or #2 were diluted in low BSA blocking buffer to 1ng/ml and incubated on MTP. After incubation and wash, AP substrate was added to each well and Relative Luminescence Unit (RLU) was measured by a plate reader. Modulations for each antibody were calculated using RLU of each coating concentration level divided by the RLU of background (buffer blank, no antibody coating).

Results:

Antibodies #1 to #12 showed modulations. Antibody #13 didn't show any response. Antibodies #2, #5, #7, #10 and #12 gave good modulations and were proceeded to screen with DHEAS competition.

Table [SEQ Table * ARABIC]: Antibodies screened on MTP

Anti-DHEAS antibody #	Name	Supplier	Cat#	Lot#	McAb clone#
1	anti-DHEAS McAb	LS Bio	LS-C55756	27911	1177/1122
2	anti-DHEAS McAb	LS Bio	LS-C55788	27865	1-15.1
3	anti-DHEAS McAb	LS Bio	LS-C55789	38101	1177/1241
4	anti-DHEAS McAb	LS Bio	LS-C128593	38186	
5	anti-DHEAS McAb	LS Bio	LS-C128594	38198	
6	anti-DHEAS McAb	LS Bio	LS-C128595	32885	
7	anti-DHEAS McAb	antibody-online	ABIN594679	(not provided by vendor)	
8	anti-DHEAS McAb	antibody-online	ABIN594680	(not provided by vendor)	
9	anti-DHEAS McAb	US Biological	D3225-25	L12013002	10B2720
10	anti-DHEAS McAb	US Biological	D3225-25A	L11052464	10B2721
11	anti-DHEAS McAb	US Biological	D3225-25B	L12083064	10B2722
12	Rabbit anti-DHEAS PcAb	LS Bio	LS-C85783	32884	
13	Sheep anti-DHEAS PcAb	GenWay	GWB-588692	1006	

Table [SEQ Table * ARABIC]: Results of initial screen on MTP

	Ab#1	Ab#2	Ab#3	Ab#4	Ab#5	Ab#6	Ab#7	Ab#8	Ab#9	Ab# 10	Ab# 11	Ab# 12	Ab# 13
AP conj#1													
AP conj#2													

modulation >10
modulation between 2 to 10
no modulation

2.1.2 Antibody screening with DHEAS competition

After initial screening, antibodies #2, #5, #7, #10 and #12 were tested on MTP for DHEAS and DHEAS-AP conjugate competitively binding to antibody.

Methods:

The MTP was coated with secondary antibodies at 20ug/ml in coating buffer. Antibodies #2, #5, #7 and #19 were prepared in blocking buffer at 5ug/ml and Ab#12 was diluted 1:100000 in blocking buffer to incubate with secondary antibodies on plate. DHEAS-AP conjugates #1 or #2

were diluted in Theranos small molecule AP stabilizer to working solution at 5ng/ml. DHEAS calibrators were prepared from 100ng/ml to 100,000ng/ml in low BSA blocking buffer and then further diluted 100-fold with low BSA blocking buffer. Diluted DHEAS calibrators were mixed with DHEA-AP conjugate working solution at 1:1 volume ratio in MTP wells to incubate with coated antibodies. After incubation and wash, AP substrate was added to each well and Relative Luminescence Unit (RLU) was measured by a plate reader. Modulations for each antibody were calculated using RLU of each calibrator concentration level divided by the RLU of background (buffer blank).

DHEA was also prepared in low BSA blocking buffer at high concentrations to be tested for cross reactivity.

Results:

All five antibodies showed DHEAS competition but all had low modulations. Only Ab#12 showed reasonable trend of dose response. Cross reactivity with DHEA was seen at testing condition. Both conjugate #1 and #2 had similar response in this test. Considering that the competitive assay format might show difference between MTP and readers, all antibodies except #13 were tested on readers for screening again.

Table [SEQ Table * ARABIC]: Antibody screening with DHEAS competition

		Ab#2	Ab#5	Ab#7	Ab#10	Ab#12
	DHEAS Sample conc. (ng/ml)	**Modulation**	**Modulation**	**Modulation**	**Modulation**	**Modulation**
Conjugate #1	**100,000**	3.3	3.6	3.5	3.8	4.2
	10,000	3.4	3.7	3.6	3.3	3.2
	1000	3.0	3.1	3.0	3.1	2.0
	100	1.0	0.9	0.9	1.0	1.3
	0	1.0	1.0	1.0	1.0	1.0
	DHEA 100,000	4.5	4.9	5.0	4.8	1.7
	DHEA 1000	1.3	1.3	1.3	1.2	0.9
	DHEAS Sample conc. (ng/ml)	**Modulation**	**Modulation**	**Modulation**	**Modulation**	**Modulation**
Conjugate #2	**100,000**	3.7	4.0	3.8	2.7	1.4
	10,000	3.9	4.0	3.9	3.4	1.7
	1000	3.0	3.1	3.2	2.7	1.2
	100	1.4	1.3	1.3	1.3	1.1
	0	1.0	1.0	1.0	1.0	1.0
	DHEA 100,000	3.5	3.4	3.5	3.2	1.2
	DHEA 1000	1.8	1.7	1.5	1.6	0.9

Table [SEQ Table * ARABIC]: DHEAS-AP conjugates used in antibody screening

Conjugate #	Supplier	Lot#	Linker
DHEAS-AP conjugate #1	Theranos in-house	DHEAS-BMPH-AP_01_010813	BMPH
DHEAS-AP conjugate #2	Theranos in-house	DHEAS-EMCH-AP_01_010813	EMCH

2.2 Antibody screen on readers

For better screening of antibodies in final assay platform, antibodies #1 to #12 were screened for conjugate direct binding and DHEAS competition on Edison readers after the initial screen on MTP. Selected antibodies were also tested for cross reactivity and interference with similar structure compounds on readers

2.2.1 Antibody evaluation on readers with antibody-conjugate direct binding

Methods:

Tips were coated with UA at 20ug/ml in coating buffer and then biotin labeled secondary antibody at 10ug/ml in blocking buffer. Antibodies #1 to #11 were prepared in blocking buffer at 1ug/ml or Ab#12 at 1:100000 dilutions in blocking buffer. Antibody solutions were incubated with coated tips first. Conjugate #1 or #2 were diluted to different concentration in Theranos small molecule AP stabilizer and reacted with tips in the second incubation. Edison protocol Generic2_ND was used for direct binding.

Results:

All antibodies showed higher modulation on readers than on MTP. Top three antibodies, Ab#2, #5, #7, and #10 were chosen for the next step evaluation. Ab#12 showed least cross reactivity to other compounds on MTP, so Ab#12 was also included for screening with DHEAS competition. Both conjugate #1 and #2 worked to bind to antibodies. Conjugate #2 showed higher response to all antibodies than conjugate #1. Conjugate #2 was selected for further use.

Table [SEQ Table * ARABIC]: Antibody direct binding to conjugate-1 on readers

	Ab1			Ab2			Ab3		
Conj. conc.	**Mean RLU**	**%CV**	**Modulation**	**Mean RLU**	**%CV**	**Modulation**	**Mean RLU**	**%CV**	**Modulation**
Conj. 100ng/ml	6465	24	27	47259	3	77	4533	25	33
Conj. 10ng/ml	724	6	3	4041	22	7	539	12	4
Conj. 1ng/ml	235	4	1	616	9	1	135	36	1
	Ab4			**Ab5**			**Ab6**		

Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	6535	7	41	53768	14	79	5062	5	23
Conj. 10ng/ml	636	23	4	5203	13	8	497	8	2
Conj. 1ng/ml	161	41	1	682	0	1	217	5	1
	Ab7			Ab8			Ab9		
Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	25180	16	66	3017	15	20	4533	25	33
Conj. 10ng/ml	3315	47	9	450	6	3	539	12	4
Conj. 1ng/ml	383	10	1	148	24	1	135	36	1
	Ab10			Ab11			Ab12		
Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	16929	9	46	1886	20	9	20746	22	19
Conj. 10ng/ml	1756	1	5	214	7	1	4086	20	4
Conj. 1ng/ml	366	34	1	205	48	1	1067	4	1

Table [SEQ Table * ARABIC]: Antibody direct binding to conjugate-2 on readers

	Ab1			Ab2			Ab3		
Conj. Conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	15802	5	52	256794	1	106	11323	14	43
Conj. 10ng/ml	2174	15	7	26610	15	11	1044	9	4
Conj. 1ng/ml	307	16	1	2415	4	1	266	21	1
	Ab4			Ab5			Ab6		
Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	12987	2	80	252217	9	106	8643	22	59
Conj. 10ng/ml	1420	15	9	33588	14	14	1212	17	8
Conj. 1ng/ml	163	51	1	2373	36	1	147	30	1
	Ab7			Ab8			Ab9		

Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	142584	5	60	4814	27	50	7241	12	62
Conj. 10ng/ml	27286	26	11	533	30	6	537	2	5
Conj. 1ng/ml	2396	35	1	96	1	1	116	24	1
	Ab10			Ab11			Ab12		
Conj. conc.	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
Conj. 100ng/ml	109418	3	79	3491	2	37	55125	8	34
Conj. 10ng/ml	14367	7	10	447	3	5	8685	9	5
Conj. 1ng/ml	1377	6	1	94	38	1	1628	2	1

2.2.2 Antibody screening on readers with DHEAS competition

Methods:

DHEAS competition was evaluated using similar procedure as antibody-conjugate direct binding. Ab#2, #5 and #7 were prepared at 5ug/ml and Ab#12 was diluted 1:10000 in blocking buffer. DHEAS was prepared in low BSA assay buffer at a series of concentrations and mixed with conjugate at 1:50 dilution. The mixture was then loaded to cartridges.

Results:

All four antibodies showed DHEAS competition curve. Therefore all of them were proceeded to be tested for cross reactivity with other compounds.

Table [SEQ Table * ARABIC]: DHEAS competition on readers

		Ab#2			Ab#5		
Calibrator	DHEAS Conc.(ng/ml)	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
1	10000	2747	13.1	30.9	2787	10.1	30.7
2	4000	4250	20.9	20.0	3770	3.2	22.7
3	1600	4604	8.1	18.4	4288	2.6	19.9
4	640	9453	10.3	9.0	8844	23.8	9.7
5	256	24270	18.3	3.5	26784	19.7	3.2
6	102	48096	0.3	1.8	49423	3.9	1.7
7	41	64661	14.3	1.3	63978	10.9	1.3
8	0	84799	14.0	1.0	85487	12.7	1.0

		Ab#7			Ab#12		
Calibrator	Conc.(ng/ml)	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
1	10000	2876	22.2	32.9	8162	25.6	15.0
2	4000	4198	23.1	22.5	17892	13.4	6.9
3	1600	4925	2.8	19.2	33191	24.4	3.7
4	640	9884	20.5	9.6	61718	15.6	2.0
5	256	26566	16.3	3.6	93155	4.7	1.3
6	102	53714	2.8	1.8	112729	14.8	1.1
7	41	67924	11.5	1.4	128941	13.2	1.0
8	0	94577	15.1	1.0	122686	19.2	1.0

2.2.3 Cross reactivity screening

Several compounds with similar structure to DHEAS were tested for cross reactivity.

Methods:

Eight compounds were prepared at significant high concentrations and were tested using the same procedure as DHEAS competition. All compounds were calculated using the dose-response curve of DHEAS competition to estimate the percentage of cross reactivity with each antibody.

Results

Antibodies #2, #5 and #7 had cross reactivity to DHEA at about 15-20% and moderate cross reactivity to Hydrocortisone, 17-a-Hydroxyprogensterone, Prednisolone and Testosterone at less than 5%. No cross reactivity showed with antibodies #2, #5 and #7 to Estrone, Estriol and Estradiol. Antibody #12 didn't show cross reactivity to any compound tested.

Table [SEQ Table * ARABIC]: Cross reactivity screening

		Ab#2				Ab#5			
Sample	Conc. (ng/ml)	Mean RLU	%CV	Cal conc. (ng/ml)	% Cross reactivity	Mean RLU	%CV	Cal conc. (ng/ml)	% Cross reactivity
DHEA	4000	8622	6.0	734.3	18.4	8723	12.2	610.6	15.3
hydrocortisone	10000	23324	5.6	275.9	2.8	23737	11.6	313.9	3.1
17-a-Hydroxyprogen sterone	10000	11812	15.0	521.4	5.2	13486	12.1	447.3	4.5
Prednisolone	10000	44577	25.0	107.7	1.1	53983	10.0	76.1	0.8
Testosterone	10000	20309	7.9	317.1	3.2	21430	5.9	340.2	3.4
Estrone (E1)	10000	79176	2.4	OORL	--	80292	5.6	OORL	--
Estriol (E3)	10000	77385	17.4	OORL	--	80249	14.1	OORL	--

Estradiol (E2)	10000	62050	5.5	OORL	--	67761	11.4	OORL	--
		Ab#7				**Ab#12**			
Sample	**Conc. (ng/ml)**	**Mean RLU**	**%CV**	**Cal conc. (ng/ml)**	**% Cross reactivity**	**Mean RLU**	**%CV**	**Cal conc. (ng/ml)**	**% Cross reactivity**
DHEA	4000	8658	10.8	778.0	19.4	114160	6.3	OORL	--
hydrocortisone	10000	20895	11.6	357.7	3.6	106701	16.8	OORL	--
17-a-Hydroxyprogensterone	10000	13962	10.3	491.9	4.9	114991	16.5	OORL	--
Prednisolone	10000	54120	17.3	89.5	0.9	123500	9.3	OORL	--
Testosterone	10000	19482	9.6	379.0	3.8	100057	17.1	OORL	--
Estrone (E1)	10000	84082	1.3	OORL	--	103738	24.2	OORL	--
Estriol (E3)	10000	80932	13.3	OORL	--	131197	7.4	OORL	--
Estradiol (E2)	10000	70479	12.3	OORL	--	121419	12.7	OORL	--

2.2.4 Interference test

Interference tests were conducted for compounds which showed cross reactivity to antibodies Ab#2, #5 and #7.

Methods:

For compounds showing cross reactivity, interference tests were performed by first making matrix with these compounds at the concentrations three times higher than their clinical concentrations and then spiking DHEAS into matrix. These spiking samples were analyzed using the same procedure and the DHEAS concentrations were calculated from DHEAS competition dose response curve. The recovery of calculated results vs. nominal concentration was compared to evaluate the interference of these compounds on DHEAS measurement.

Results:

All of DHEA, Hydrocortisone, 17-a-Hydroxyprogensterone, Prednisolone and Testosterone showed certain levels interference on DHEAS measurement with antibodies #2, #5 and #7. Because the matrix had three times higher concentration of highest clinical level of these testing compounds, the interferences were not considered as severe. All three antibodies were further tested with clinical samples.

Table [SEQ Table * ARABIC]: Interference test

		Ab#2		Ab#5		Ab#7	
Interference compound (matrix at 3x of highest clinical levels)	**DHEAS spiked Conc. (ng/ml)**	**DHEAS back cal conc. (ng/ml)**	**% accuracy**	**DHEAS back cal conc. (ng/ml)**	**% accuracy**	**DHEAS back cal conc. (ng/ml)**	**% accuracy**
Interference #1 DHEA 120ng/ml	4000	2797	70	5166	129	4715	118
Interference #1 DHEA 120ng/ml	640	572	89	709	111	663	104
Interference #1 DHEA 120ng/ml	102	158	155	149	146	172	169
Interference #1 DHEA 120ng/ml	0	57	--	66	--	93	--
Interference #2 Hydrocortisone 750ng/ml	4000	1175	29	1822	46	3308	83
Interference #2 Hydrocortisone 750ng/ml	640	570	89	746	117	728	114
Interference #2 Hydrocortisone 750ng/ml	102	166	163	191	187	201	197
Interference #2 Hydrocortisone 750ng/ml	0	24	--	51	--	81	--
Interference #3 17-a-Hydroxyprogesterone 30ng/ml	4000	1719	43	15473	387	1811	45
Interference #3 17-a-Hydroxyprogesterone 30ng/ml	640	567	89	766	120	783	122
Interference #3 17-a-Hydroxyprogesterone 30ng/ml	102	186	182	646	633	185	181
Interference #3 17-a-Hydroxyprogesterone 30ng/ml	0	39	--	415	--	92	--
Interference #4 Prednisolone 750ng/ml	4000	2302	58	3550	89	4082	102
Interference #4 Prednisolone 750ng/ml	640	571	89	702	110	569	89
Interference #4 Prednisolone 750ng/ml	102	137	134	119	117	176	173
Interference #4 Prednisolone 750ng/ml	0	33	--	39	--	63	--
Interference #5 Testosterone 40ng/ml	4000	2933	73	3928	98	5407	135
Interference #5 Testosterone 40ng/ml	640	573	90	665	104	610	95

Interference #5 Testosterone 40ng/ml	102	94	92	131	129	149	146
Interference #5 Testosterone 40ng/ml	0	26	--	32	--	110	--

2.2.5 Training set

"Training set" containing twelve clinical samples were analyzed by reference method and with antibodies #2, #5, #7 and #12 for DHEAS concentration to evaluate the preliminary correlation for antibody selection.

Methods:

In the preliminary test, Edison protocol Generic2_ND was used with sample pre-diluted before loading to cartridges. Tips were coated with UA and Biotin labeled secondary antibody. Ab#2, #5, and #7 were diluted to 5ug/ml and Ab#12 was diluted 1:10000 in blocking buffer. DHEAS calibrators were prepared in low BSA assay buffer. DHEAS-AP conjugate#2 was prepared in Theranos small molecule AP stabilizer at 500ng/ml as 10x stock solution. Calibrators or samples were diluted 1:50 with low BSA assay buffer and conjugate was mixed into diluted samples to the final concentration of 50ng/ml. Tips reacted with primary antibodies in the first incubation and with sample/conjugate mixture in the second incubation.

Results:

Antibodies #2, #5 and #7 didn't show reasonable correlation to the results obtained by SIEMENS immunlite analysis as reference method from CLIA lab. Ab#12 had good recovery and correlation to reference method. Therefore Ab#12 was chosen for further optimization.

Table [SEQ Table * ARABIC]: Training set

		Ab#2				Ab#5			
Sample ID	**Conc. from CLIA (ng/ml)**	**Mean RLU**	**%CV**	**back cal conc. (ng/ml)**	**% accuracy**	**Mean RLU**	**%CV**	**back cal conc. (ng/ml)**	**% accuracy**
Pediatrics 9	**183**	19159	36.5	431.0	236	18324	31.3	571.0	312
Pediatrics 21	**<150**	25855	20.6	228.0	--	8264	78.2	839.3	--
PCOS 1	**1510**	2652	29.4	20116.0	1332	1415	13.3	OORH	--
PCOS 5	**2670**	2732	15.2	16297.9	610	2492	11.8	19205.5	719
PCOS 7	**663**	12445	0.5	581.4	88	13387	11.8	726.2	110
PCOS 17	**4640**	4069	18.4	2082.5	45	4342	8.4	1857.0	40
M2	**989**	25855	20.6	228.0	23	4921	16.5	1414.8	143
M3	**2000**	2652	29.4	20116.0	1006	3444	28.1	3833.0	192
M5	**298**	2732	15.2	16297.9	5469	8496	10.8	830.5	279

F2	**262**	4069	18.4	2082.5	795	13586	9.4	721.4	275
F3	**154**	12445	0.5	581.4	378	9988	37.8	792.4	515
F4	**617**	4069	18.4	2082.5	338	7032	8.3	914.1	148
		Ab#7				**Ab#12**			
Sample ID	**Conc. from CLIA (ng/ml)**	**Mean RLU**	**%CV**	**back cal conc. (ng/ml)**	**% accuracy**	**Mean RLU**	**%CV**	**back cal conc. (ng/ml)**	**% accuracy**
Pediatrics 9	**183**	7178	53.9	1238.2	677	93800	7.9	159.0	87
Pediatrics 21	**<150**	3784	30.2	5040.1	3360	100697	10.8	103.7	--
PCOS 1	**1510**	1642	11.3	OORH	--	47439	8.6	1583.3	105
PCOS 5	**2670**	2810	10.5	17618.2	660	30992	9.8	2607.0	98
PCOS 7	**663**	10691	17.9	843.9	127	60201	21.9	958.3	145
PCOS 17	**4640**	4829	11.2	2491.3	54	18987	17.2	4820.2	104
M2	**989**	4655	7.4	2726.9	276	47724	3.3	1567.7	159
M3	**2000**	3582	22.9	6125.5	306	33920	2.1	2393.1	120
M5	**298**	8208	3.3	1064.6	357	72254	1.0	538.0	181
F2	**262**	14694	14.1	650.9	248	82014	7.5	317.6	121
F3	**154**	10325	18.0	867.6	563	85625	2.8	258.4	168
F4	**617**	7134	15.3	1247.8	202	57511	4.2	1076.0	174

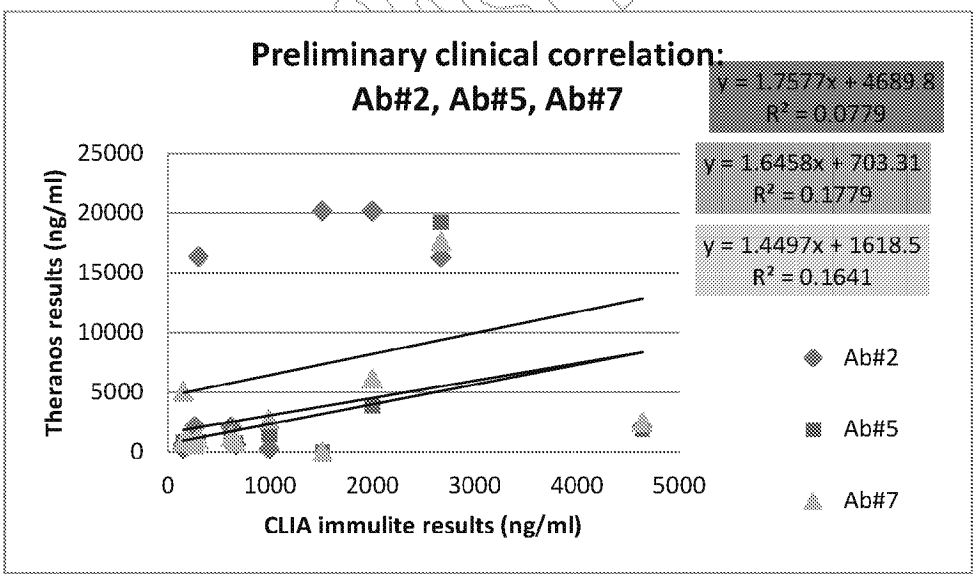


Figure [SEQ Figure * ARABIC]: Preliminary clinical sample correlation of Ab#2 #5 and #7

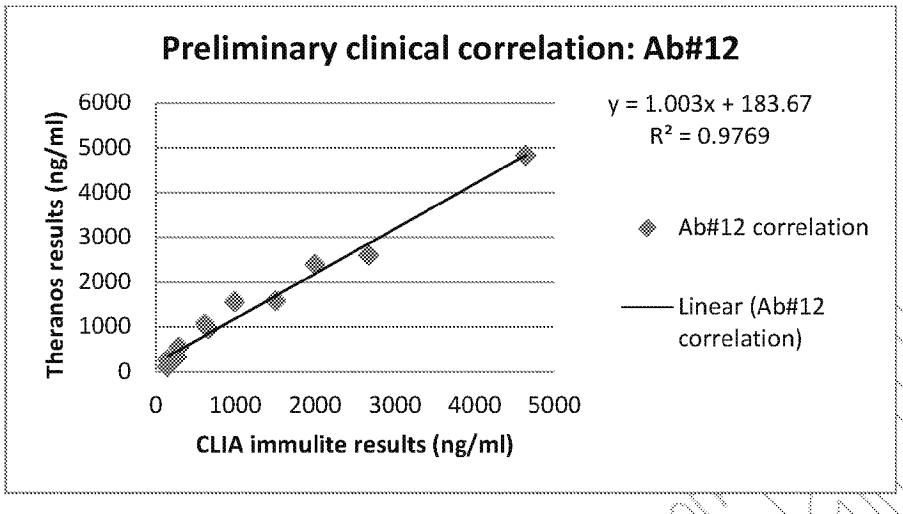


Figure [SEQ Figure * ARABIC]: Preliminary clinical sample correlation of Ab#12

2.3 Assay condition optimization

Ab#12 was chosen as the capture antibody. Further optimization was done to finalize assay conditions.

2.3.1 Optimization of sample dilution

In the preliminary assay condition, calibrators and samples were analyzed at 50-fold dilution. While calibration curve showed acceptable sensitivity, the overall modulation was considered low to give enough resolution across the calibration range. Sample dilution factors were first compared to choose better modulation. Several clinical samples were tested with calibrators to evaluate matrix effect at different sample dilutions.

Methods:

Assay format of tips with UA and Biotin labeled secondary antibody and capture Ab in solution was kept. Samples were pre-diluted at 1:5, 1:10, or 1:25 and mixed with DHEAS-AP conjugate. Clinical samples were calculated form the calibration curve which had the same dilution factor and recovery was compared at each level of dilution.

Results:

Calibration curves at different sample dilutions showed high sensitivity and good curve regression. The lower sample dilution, the higher modulation of the whole curve was obtained. Although calibration curve was good at any dilution level, clinical samples showed the trend of lower recovery at lower dilution level. To minimize the matrix effect, calibrators were prepared in steroid depleted serum and analyzed to evaluate clinical sample recovery.

Table [SEQ Table * ARABIC]: Calibration curve at sample dilution 1:5

		Ab#12: Sample dilution 1:5				
Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	1513	21.7	71.9	9815	98
2	4000	3178	13.2	34.2	4137	103
3	1600	8663	3.4	12.6	1571	98
4	640	19470	20.5	5.6	609	95
5	256	33500	23.2	3.2	255	100
6	102	50273	3.1	2.2	110	108
7	41	75710	11.7	1.4	39	95
8	0	108739	9.7	1.0		

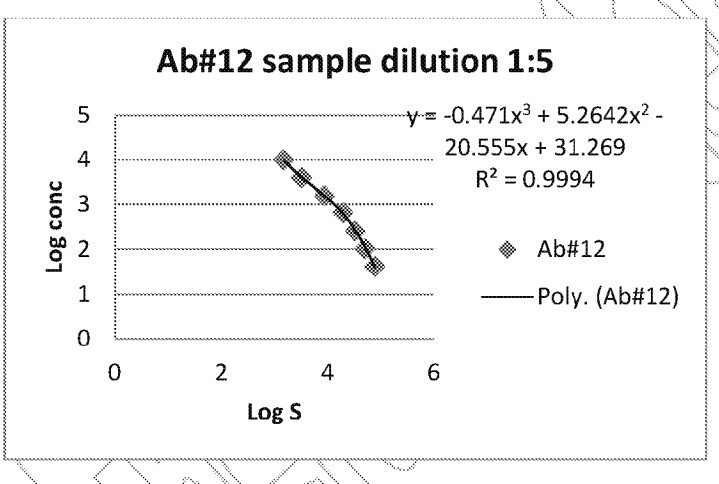


Figure [SEQ Figure * ARABIC]: Calibration curve at sample dilution 1:5

Table [SEQ Table * ARABIC]: Calibration curve at sample dilution 1:10

		Ab#12: Sample dilution 1:10				
Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc (ng/ml)	% accuracy
1	10000	2642	10.7	45.2	10131	101
2	4000	5843	20.3	20.4	4056	101
3	1600	16287	3.7	7.3	1629	102
4	640	32000	9.3	3.7	660	103
5	256	51040	16.0	2.3	254	99
6	102	69980	10.1	1.7	108	105
7	41	93028	7.9	1.3	42	101
8	0	119446	3.8	1.0		

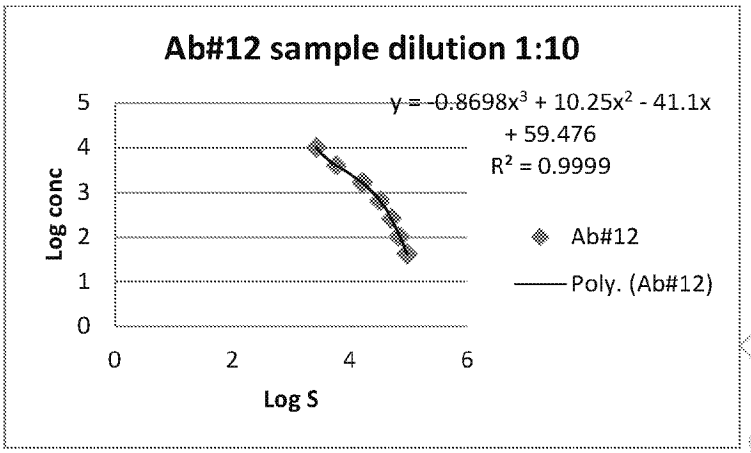


Figure [SEQ Figure * ARABIC]: Calibration curve at sample dilution 1:10

Table [SEQ Table * ARABIC]: Calibration curve at sample dilution 1:25

		Ab#12: Sample dilution 1:25				
Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	6253	4.5	18.5	10174	102
2	4000	14823	10.7	7.8	3737	93
3	1600	34319	8.7	3.4	1926	120
4	640	58061	7.6	2.0	527	82
5	256	68076	15.4	1.7	280	109
6	102	84259	4.9	1.4	96	94
7	41	95321	24.2	1.2	45	110
8	0	115787	9.4	1.0		

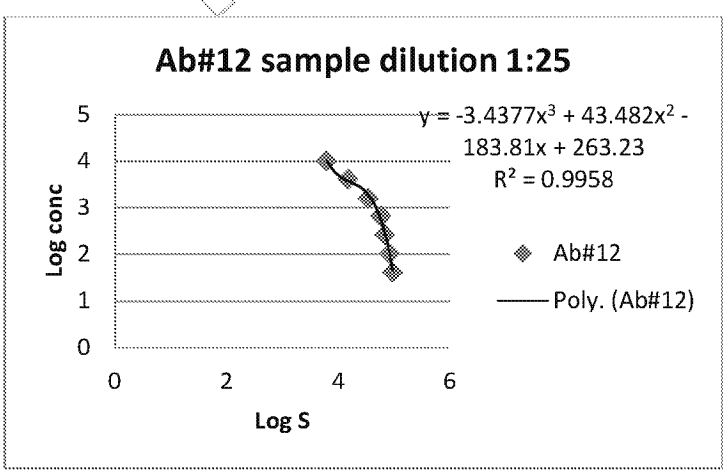


Figure [SEQ Figure * ARABIC]: Calibration curve at sample dilution 1:25

Table [SEQ Table * ARABIC]: Clinical samples analyzed at different dilutions

	Sample dilution 1:5				
Sample ID	Conc. from CLIA (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Pediatrics 9	183	36706	18.7	214.3	117.1
Pediatrics 21	<150	39948	23.3	181.2	--
PCOS 1	1510	15948	2.8	793.6	52.6
PCOS 5	2670	8291	23.4	1641.8	61.5
PCOS 7	663	19159	16.3	622.8	93.9
PCOS 17	4640	4050	6.9	3257.2	70.2
	Sample dilution 1:10				
Sample ID	Conc. from CLIA (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Pediatrics 9	183	52233	6.5	239.7	131.0
Pediatrics 21	<150	56566	16.1	195.8	--
PCOS 1	1510	22980	27.5	1086.4	71.9
PCOS 5	2670	14472	4.4	1835.9	68.8
PCOS 7	663	29021	8.3	774.4	116.8
PCOS 17	4640	7873	4.9	3133.8	67.5
	Sample dilution 1:25				
Sample ID	Conc. from CLIA (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Pediatrics 9	183	81491	10.0	115.4	63.0
Pediatrics 21	<150	79155	19.8	134.9	89.9
PCOS 1	1510	40261	7.0	1449.7	96.0
PCOS 5	2670	21796	12.3	3056.6	114.5
PCOS 7	663	37172	4.4	1687.8	254.6
PCOS 17	4640	12927	7.7	4024.6	86.7

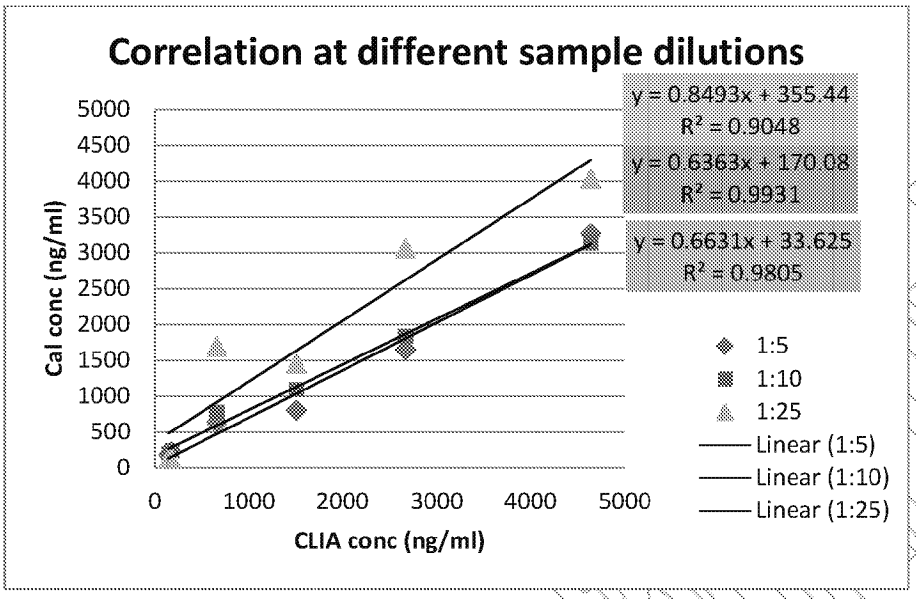


Figure [SEQ Figure * ARABIC]: Correlation at different sample dilutions

2.3.2 Calibrators in steroid depleted serum

Methods:

DHEAS calibrators were prepared in steroid depleted serum and analyzed at 1:5 or 1:10. Clinical samples were also analyzed and calculated from depleted serum calibration curve.

Results:

Comparing to calibrator curve prepared in low BSA assay buffer, calibration curve in depleted serum showed unparalleled signal shift. Clinical samples calculated from depleted serum curve didn't show good recovery. It indicated that steroid depleted serum might contain low level of DHEAS but the concentration could not be determined precisely by reference method due to the sensitivity of reference method. As calibrators were preferred to be prepared in assay buffer, sample dilution of 1:25 was chosen to minimize the matrix effect.

Table [SEQ Table * ARABIC]: Calibrators prepared in steroid depleted serum

		Sample dilution 1:5			Sample dilution 1:10		
Sample ID	Conc. from CLIA (ng/ml)	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
1	10000	2770	4.1	14.4	5123	1.6	12.2
2	4000	6286	25.5	6.4	7013	1.7	8.9
3	1600	11761	23.7	3.4	18712	13.4	3.3
4	640	20440	7.3	2.0	31329	19.5	2.0

5	256	26035	3.6	1.5	39775	7.0	1.6
6	102	24375	10.2	1.6	48307	3.3	1.3
7	41	34906	1.3	1.1	51129	6.1	1.2
8	0	39936	22.0	1.0	62597	14.6	1.0

Table [SEQ Table * ARABIC]: Clinical samples analyzed at 1:10 and calculate from depleted serum calibration curve

Sample ID	Conc. from CLIA (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Pediatrics 9	183	52233	6.5	44.2	24.2
Pediatrics 21	<150	56566	16.1	23.1	--
PCOS 1	1510	22980	27.5	1322.2	87.6
PCOS 5	2670	14472	4.4	1913.5	71.7
PCOS 7	663	29021	8.3	818.3	123.4
PCOS 17	4640	7873	4.9	3033.1	65.4

2.3.3 Comparison of assay format

2.3.3.1 Comparison of capture format

In early stage development, the assay format was used as secondary antibody coating and capture antibody in solution format in order to screen antibodies. To choose the final Edison protocol, capture format was compared. Sample dilution was also needed to be transferred from pre-dilution to on board dilution.

Methods:

Two coating formats were compared. "Antibody in solution" used UA and biotin labeled goat anti-rabbit IgG coated tips. In "capture on tip" format, capture antibody Ab#12 was coated on tip in a "3-stack" format which had UA first and then biotin labeled goat anti-rabbit IgG and Ab#12 as the third layer of coating. The same protocol Generic2_25x_coincubation was used to compare these two formats to keep on-board sample dilution 1:25.

Results:

"Capture on tip" format gave much higher modulation so it showed advantage to potentially increase sensitivity after further optimization.

Table [SEQ Table * ARABIC]: Comparison of assay format

Format	Capture Ab in solution	Capture Ab on tip

Protocol		Generic2_25x_coincubation			Generic2_25x_coincubation		
tip		UA + Biotin Goat anti-rabbit IgG			UA + Biotin Goat anti-rabbit IgG + Ab12		
capture Ab		Ab12 in solution			Ab12 on tip		
capture final conc.		1:20000			1:10000		
sample dilution		25x			25x		
detection final conc.		25ng/ml			25ng/ml		
Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Mean RLU	%CV	Modulation
1	10000	6974	6.4	16.7	2478	14.0	31.1
2	4000	14808	15.8	7.9	6562	7.7	11.7
3	1600	31955	5.9	3.6	14756	22.1	5.2
4	640	52433	10.3	2.2	25677	12.6	3.0
5	256	58748	20.5	2.0	42556	3.3	1.8
6	102	81803	18.7	1.4	60275	14.1	1.3
7	41	86093	4.4	1.4	72081	4.9	1.1
8	0	116470	5.5	1.0	76935	11.7	1.0

2.3.3.2 Confirm "capture on tip" format with Bio Rad controls

Methods:

In order to confirm the assay performance of "capture on tip" format, Bio Rad Lyphochek Immunoassay Plus Controls were analyzed using "capture on tip" format. Although these controls had reported concentration range, they were also sent to CLIA lab for analysis with reference method to calculate accuracy precisely.

Results:

Bio Rad controls gave reasonable recovery in this format. "Capture on tip" format was chosen for further optimization.

Table [SEQ Table * ARABIC]: Bio Rad controls analyzed by "capture on tip" assay format

Sample ID	Conc. (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Bio Rad Control 371	652	26401	11.2	668.5	102.5
Bio Rad Control 372	1300	17589	11.0	1288.6	99.1
Bio Rad Control 373	4310	7735	7.6	3225.0	74.8

2.3.4 Detection conjugate titration

Methods:

Titration of detection conjugate concentration was done by preparing detection conjugate in Theranos in-house AP stabilizer at 12.5ng/ml, 25ng/ml and 50ng/ml respectively. Capture antibody was coated on tip and the same Edison protocol Genric2_25x_coincubation was used.

Results:

Detection conjugate showed signal saturation at 50ng/ml. Signal of 12.5ng/ml detection conjugate seemed to be low. 25ng/ml of detection conjugate gave the best modulation so it was chosen as final condition.

Table [SEQ Table * ARABIC]: Results of detection conjugate titration

Detection conjugate conc.		50ng/ml			25ng/ml			12.5ng/ml		
Calibrator	Conc. (ng/ml)	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation
1	10000	7011	2.8	21.4	1875	6.5	47.1	923	26.0	42.7
2	4000	18395	0.2	8.2	4663	3.4	18.9	2052	5.8	19.2
3	1600	31976	18.1	4.7	9936	14.8	8.9	4240	15.8	9.3
4	640	56617	16.3	2.6	21421	20.4	4.1	9040	40.9	4.4
5	256	95064	15.0	1.6	43689	27.1	2.0	17910	17.3	2.2
6	102	108841	4.2	1.4	61239	10.7	1.4	29210	17.2	1.3
7	41	105490	17.4	1.4	77751	8.1	1.1	32086	6.5	1.2
8	0	149968	8.2	1.0	88269	12.3	1.0	39405	12.6	1.0

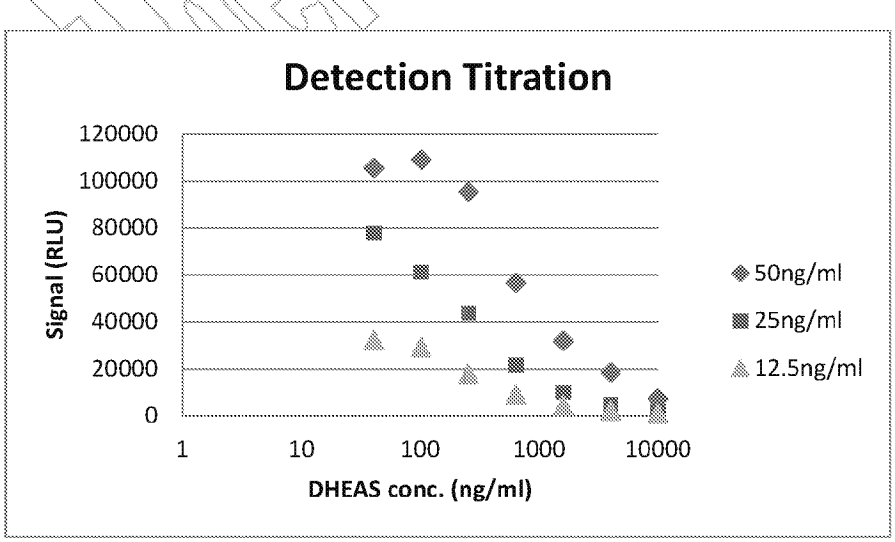


Figure [SEQ Figure * ARABIC]: Detection conjugate titration

2.3.5 Selection of detection conjugate stabilizer

Methods:

With detection conjugate concentration at 25ng/ml, detection conjugate was prepared in Sigma BioStab AP stabilizer, or Surmodics StabilZyme AP stabilizer, or Theranos in-house AP stabilizer. All conditions were tested with protocol Generic2_25x_coincubation to compare the effect of AP stabilizers.

Results:

Detection conjugate in three different stabilizers gave similar modulation. However, among three AP stabilizers, StabilZyme showed the best sensitivity in terms of better signal separation at lower concentration portion in the calibration range. Stabilzyme AP stabilizer was chosen as final reagent for detection conjugate.

Table [SEQ Table * ARABIC]: Results of detection conjugate stabilizer comparison

AP stabilizer		Theranos in-house stabilizer			StabliZyme			BioStab		
Calibrator	Conc. (ng/ml)	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation
1	10000	1875	6.5	47.1	2635	21.6	55.7	3199	10.5	51.6
2	4000	4663	3.4	18.9	7210	10.9	20.3	5117	8.7	32.3
3	1600	9936	14.8	8.9	18947	16.7	7.7	12957	7.0	12.8
4	640	21421	20.4	4.1	35861	2.5	4.1	31621	8.3	5.2
5	256	43689	27.1	2.0	53190	2.0	2.8	52825	16.2	3.1
6	102	61239	10.7	1.4	79979	34.0	1.8	79994	22.2	2.1
7	41	77751	8.1	1.1	95983	12.0	1.5	115910	10.2	1.4
8	0	88269	12.3	1.0	146666	10.3	1.0	165219	14.6	1.0

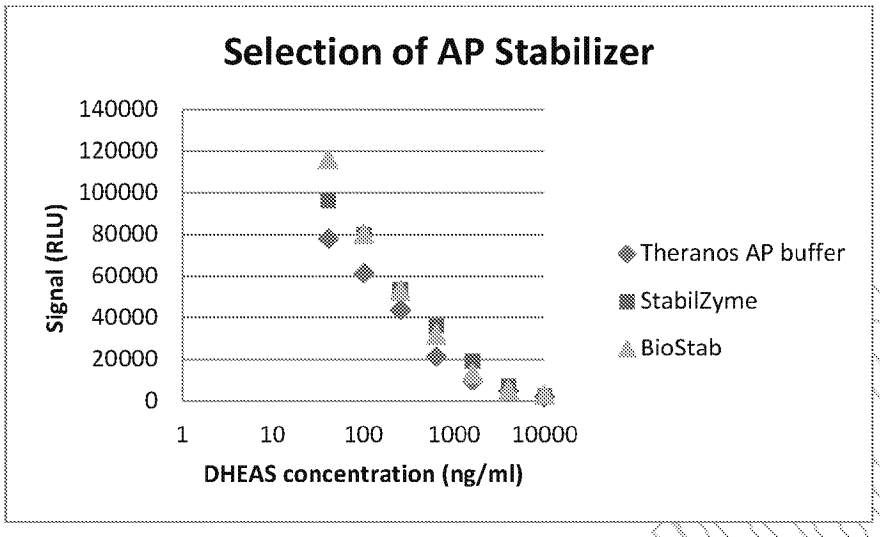


Figure [SEQ Figure * ARABIC]: Selection of AP Stabilizer

2.3.6 Determination of capture antibody concentration

Methods:

After detection conjugate condition was chosen as 25ng/ml in Stabilzyme, capture concentration titration was done by coating Ab#12 in blocking buffer at 1:10000, 1:20000, or 1:40000 dilutions respectively. Sample dilution was kept at 25x. Edison protocol Generic2_25x_coincubation was used before the finalization of protocol.

Results:

Capture antibody at all concentrations seemed to have similar modulations and sensitivity. As Ab#12 was a polyclonal antibody, using less amount of antibody was ideal in order to keep the same lot for longer use. Capture antibody dilution was chosen at 1:40000.

Table [SEQ Table * ARABIC]: Determination of capture antibody concentration

Capture conc.		1:10000			1:20000			1:40000		
Calibrator	Conc. (ng/ml)	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation
1	10000	2635	21.6	55.7	1522	11.9	50.7	841	0.8	52.6
2	4000	7210	10.9	20.3	3106	17.8	24.8	1819	25.4	24.3
3	1600	18947	16.7	7.7	9847	16.2	7.8	5438	20.8	8.1
4	640	35861	2.5	4.1	17036	8.6	4.5	9704	15.7	4.6
5	256	53190	2.0	2.8	27437	17.3	2.8	16295	27.9	2.7
6	102	79979	34.0	1.8	40111	11.4	1.9	25719	8.1	1.7
7	41	95983	12.0	1.5	58221	18.7	1.3	36633	11.1	1.2
8	0	146666	10.3	1.0	77182	12.8	1.0	44188	17.0	1.0

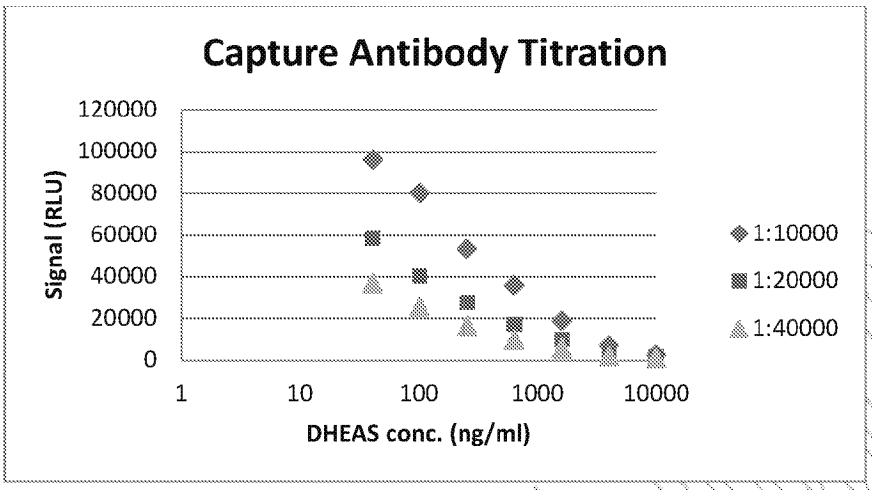


Figure [SEQ Figure * ARABIC]: Capture antibody titration

2.3.7 Re-optimization of detection conjugate concentration

Methods:

In order to keep the optimal RLU signal while using lower concentration of capture antibody, a calibration curve was generated with detection conjugate concentration being increased to 50ng/ml in StabilZyme. Edison protocols Genric2_competitive_25x_coincubation was kept.

Results:

Increasing detection conjugate concentration to 50ng/ml gave better modulation and sensitivity with capture antibody at 1:40000 dilution. This condition was chosen to analyze samples to evaluate other factors such as interfering matrix etc.

Table [SEQ Table * ARABIC]: Increasing detection conjugate concentration

		Ab 1:40000, Detection conjugate 50ng/ml				
Calibrator	**Conc. (ng/ml)**	**Mean RLU**	**%CV**	**Modulation**	**Back cal conc. (ng/ml)**	**% Accuracy**
1	10000	1586	17.1	55.9	10208.9	102.1
2	4000	3691	24.9	24.0	3738.3	93.5
3	1600	8002	8.9	11.1	1760.7	110.0
4	640	17833	18.1	5.0	639.5	99.9
5	256	30440	11.8	2.9	233.9	91.4
6	102	41276	13.9	2.1	110.9	108.7
7	41	57599	23.8	1.5	41.1	100.2
8	0	88592	7.0	1.0		

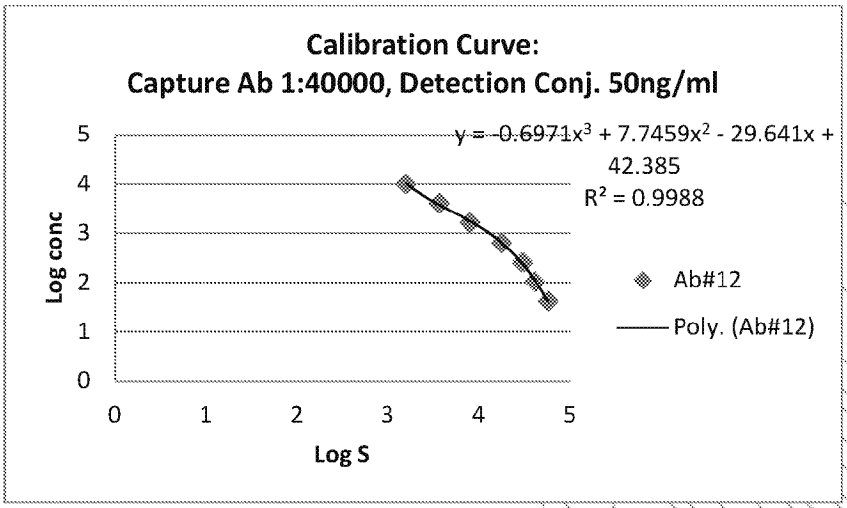


Figure [SEQ Figure * ARABIC]: Calibration curve with increased detection conjugate concentration

2.3.8 Comparison of incubation time

Methods:

Sample and substrate incubation time was tested using capture antibody 1:20000 dilution and detection conjugate concentration 25ng/ml before the re-optimization of capture and detection conditions. Edison protocols Generic2_25x_coincubation (for 10_10 incubation), Generic2_25x_coincubation_5_5, and Generic2_25x_coincubation_2_1 were compared.

Results:

Incubation 5_5 or 2_1 gave good modulation and assay sensitivity too. However, if incubation time 5_5 or 2_1 would be used, the titration of coating concentration and detection conjugate concentration might be needed to keep the optimal signal range. Without further optimization, incubation time10_10 was chosen for analyzing more samples.

Table [SEQ Table * ARABIC]: Comparison of incubation time

Incubation time		10_10			5_5			2_1		
Calibrator	Conc. (ng/ml)	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation	Mean RLU	% CV	Modulation
1	10000	1522	11.9	50.7	488	16.9	57.9	153	14.4	26.7
2	4000	3106	17.8	24.8	1486	13.9	19.0	244	19.3	16.8
3	1600	9847	16.2	7.8	2813	17.8	10.0	583	23.3	7.0
4	640	17036	8.6	4.5	6149	22.9	4.6	830	16.9	4.9
5	256	27437	17.3	2.8	9563	5.6	3.0	1429	16.6	2.9

6	102	40111	11.4	1.9	14930	13.6	1.9	2092	9.7	2.0
7	41	58221	18.7	1.3	17596	3.8	1.6	2645	5.0	1.5
8	0	77182	12.8	1.0	28215	9.8	1.0	4087	13.1	1.0

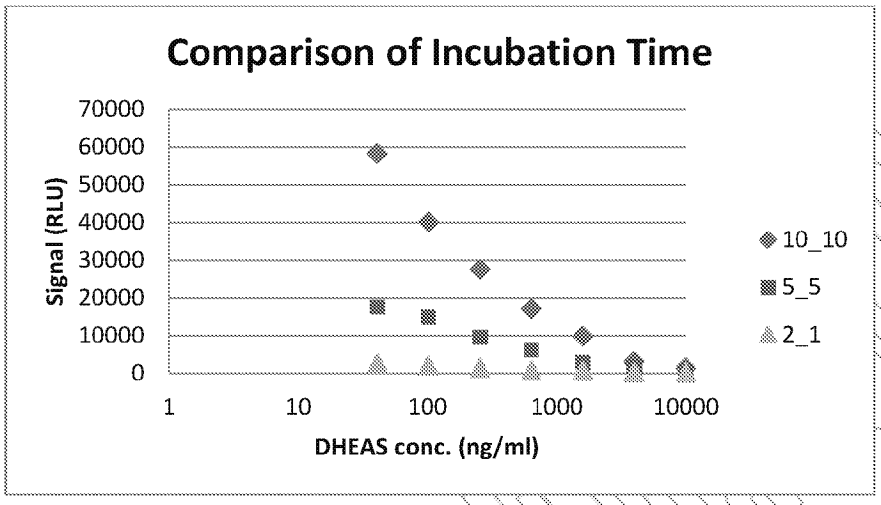


Figure [SEQ Figure * ARABIC]: Comparison of incubation time

2.4 Re-visit of assay format for better clinical sample correlation

After assay condition optimization, the procedure was set as:

- Tip coating with UA at 20ug/ml in coating buffer first, then biotin labeled goat anti-rabbit IgG 10ug/ml in blocking buffer, and then rabbit anti-DHEAS polyclonal antibody (Ab#12) at 1:40000 in blocking buffer
- Sample dilution 25-fold in Theranos low BSA assay buffer
- DHEAS-AP conjugate was mixed with diluted sample at final working concentration 50ng/ml to co-incubate with antibody
- Edison protocol: Generic2_25x_coincubation

This procedure was use to start analyzing more samples.

2.4.1 Analysis of HAMA and RF positive samples using initially finalized protocol

Five HAMA positive samples and five RF positive samples were analyzed using initially finalized protocol. However, comparing to DHEAS concentration measured by reference method, all samples showed low recovery at 40-60% in average.

To solve the problem of low recovery, more optimization effects were further done including the change of a few assay conditions and assay format.

2.4.2 Effects of trouble-shooting of low recovery

2.4.2.1 Change of sample diluent

When capture antibody concentration and detection conjugate concentration were kept unchanged, several formulations of sample diluent were tested. Under each condition, a calibration curve was generated and a set of clinical samples combining HAMA positive, RF positive, patient and health donors were analyzed. However, change of sample diluent didn't increase clinical sample recovery.

Table [SEQ Table * ARABIC]: Calibration curve and samples with SurModics protein free blocker as sample diluent

Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	1280	32.9	57.7	10231.4	102.3
2	4000	2798	16.2	26.4	3788.5	94.7
3	1600	6943	23.3	10.6	1575.5	98.5
4	640	12507	8.2	5.9	766.7	119.8
5	256	25840	17.0	2.9	192.8	75.3
6	102	31485	6.8	2.3	115.4	113.2
7	41	43346	22.5	1.7	43.1	105.1
8	0	73811	24.0	1.0		
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	11741	8.3		839.3	35.6
HAMA F1	853	13789	16.4		661.5	77.6
RF 818	504	19850	11.7		345.8	68.6
RF 822	366	20623	22.7		319.8	87.4
PCOS 1	1510	11507	19.8		863.1	57.2
PCOS 17	4640	5150	33.9		2115.6	45.6
Stanford M3 10/15/2012	2000	9113	12.9		1164.5	58.2
Stanford F3 10/15/2012	318	26439	9.9		182.3	57.3

Table [SEQ Table * ARABIC]: Calibration curve and samples with 400ug/ml of HRB-1 in Low BSA buffer as sample diluent

Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	1088	15.5	54.8	9971.3	99.7
2	4000	2470	11.5	24.1	4002.6	100.1
3	1600	5278	21.6	11.3	1631.8	102.0

4	640	10937	13.5	5.5	600.2	93.8
5	256	17886	23.2	3.3	270.6	105.7
6	102	30183	4.7	2.0	100.7	98.7
7	41	45579	16.2	1.3	40.8	99.6
8	0	59642	2.9	1.0	21.2	
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	10416	4.8		645.7	27.4
HAMA F1	853	12637	22.7		480.6	56.3
RF 818	504	15963	10.4		328.8	65.2
RF 822	366	22738	10.9		175.4	47.9
PCOS 1	1510	9716	13.6		715.3	47.4
PCOS 17	4640	5275	25.6		1633.3	35.2
Stanford M3 10/15/2012	2000	9857	25.5		700.5	35.0
Stanford F3 10/15/2012	318	21991	13.0		186.7	58.7

2.4.2.2 Re-titration of capture antibody and detection conjugate concentrations

Concentrations of capture antibody and detection conjugate were also re-titrated and tested with different buffers as sample diluent. A calibration curve was generated and a set of clinical samples combining HAMA positive, RF positive, patient and health donors were analyzed with each condition. However, the recovery of clinical samples was still low.

Table [SEQ Table * ARABIC]: Re-titration of capture antibody and detection conjugate with protein free buffer as sample diluent

Ab#12 1:20000, Conj#2 50ng/ml						
Sample diluent: Surmodics protein free buffer						
Calibrator	**Conc. (ng/ml)**	**Mean RLU**	**%CV**	**Modulation**	**Back cal conc. (ng/ml)**	**% accuracy**
1	10000	2125	35.9	56.5	10021.6	100.2
2	4000	4953	17.6	24.2	3888.0	97.2
3	1600	11834	28.2	10.1	1660.8	103.8
4	640	25178	9.8	4.8	635.5	99.3
5	256	46045	14.6	2.6	208.7	81.5
6	102	57228	5.5	2.1	125.8	123.3
7	41	87147	1.9	1.4	38.9	94.9
8	0	119977	11.8	1.0		
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**

HAMA M1	2360	15120	10.5		1262.6	53.5
HAMA F1	853	23382	13.5		710.6	83.3
RF 818	504	36048	11.8		343.7	68.2
RF 822	366	34613	30.3		370.9	101.4
PCOS 1	1510	21637	13.7		795.0	52.6
PCOS 17	4640	7928	25.2		2482.9	53.5
Stanford M3 10/15/2012	2000	17320	11.9		1070.0	53.5
Stanford F3 10/15/2012	318	48944	3.0		182.2	57.3

Table [SEQ Table * ARABIC]: Re-titration of capture antibody and detection conjugate with 400ug/ml HBR-1 in low BSA buffer as sample diluent

Ab#12 1:20000, Conj#2 50ng/ml						
Sample diluent: 400ug/ml HBR-1 in Low BSA Buffer						
Calibrator	**Conc. (ng/ml)**	**Mean RLU**	**%CV**	**Modulation**	**Back cal conc. (ng/ml)**	**% accuracy**
1	10000	1759	21.5	64.0	9999.1	100.0
2	4000	4418	26.1	25.5	4009.2	100.2
3	1600	10651	20.9	10.6	1534.3	95.9
4	640	19078	21.8	5.9	691.9	108.1
5	256	35183	9.5	3.2	240.2	93.8
6	102	52356	3.9	2.2	103.2	101.2
7	41	75796	9.4	1.5	41.2	100.4
8	0	112622	4.7	1.0	13.1	
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	13638	10.1		1117.5	47.3
HAMA F1	853	23692	2.0		490.2	57.5
RF 818	504	30021	16.3		324.3	64.3
RF 822	366	33617	10.1		262.3	71.7
PCOS 1	1510	18769	14.6		709.2	47.0
PCOS 17	4640	7685	14.7		2246.0	48.4
Stanford M3 10/15/2012	2000	16907	11.0		828.1	41.4
Stanford F3 10/15/2012	318	38505	15.4		200.7	63.1

Table [SEQ Table * ARABIC]: Re-titration of capture antibody and detection conjugate with Low BSA assay buffer as sample diluent, condition-1

Ab#12 1:20000, Conj#2 25ng/ml
Sample diluent: Low BSA buffer

Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	805	24.5	64.4	10129.4	101.3
2	4000	2264	35.4	22.9	3754.3	93.9
3	1600	5008	9.6	10.4	1716.2	107.3
4	640	10318	14.3	5.0	645.8	100.9
5	256	17123	13.5	3.0	250.3	97.8
6	102	25707	15.4	2.0	94.8	93.0
7	41	33856	19.1	1.5	43.3	105.6
8	0	51889	30.9	1.0		
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	6777	9.0		1190.4	50.4
HAMA M2	1900	6097	5.1		1361.0	71.6
HAMA M3	1270	11079	7.9		574.0	45.2
HAMA F1	853	10333	33.1		644.3	75.5
HAMA F2	1610	7119	11.0		1115.6	69.3
RF 818	504	14548	6.4		349.6	69.4
RF 822	366	16251	17.6		279.5	76.4

Table [SEQ Table * ARABIC]: Re-titration of capture antibody and detection conjugate with Low BSA assay buffer as sample diluent, condition-2

Ab#12 1:10000, Conj#2 25ng/ml						
Sample diluent: Low BSA buffer						
Calibrator	**Conc.(ng/ml)**	**Mean RLU**	**%CV**	**Modulation**	**Back cal conc. (ng/ml)**	**% accuracy**
1	10000	1418	8.5	68.7	10262.0	102.6
2	4000	3645	26.8	26.7	3655.2	91.4
3	1600	7747	5.7	12.6	1787.1	111.7
4	640	17539	6.8	5.6	662.9	103.6
5	256	32215	5.3	3.0	226.4	88.4
6	102	44221	7.5	2.2	109.7	107.5
7	41	62710	10.2	1.6	42.0	102.4
8	0	97447	11.0	1.0		
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	10241	11.8		1326.8	56.2
HAMA M2	1900	10141	12.5		1341.5	70.6

HAMA M3	1270	21912	3.9		466.6	36.7
HAMA F1	853	24295	19.9		390.4	45.8
HAMA F2	1610	12931	10.9		1005.0	62.4
RF 818	504	24276	25.4		390.9	77.6
RF 822	366	28040	10.7		299.3	81.8
RF 827	<150	50668	4.9		77.1	

2.4.2.3 Increasing sample dilution

Using different sample dilution and re-optimizing capture antibody and detection conjugate concentrations didn't improve sample recovery. This might indicate some matrix effect from insufficient sample dilutions. When using 50-fold sample dilution instead of 25-fold dilution, the recovery of clinical samples was improved to higher than 70% in average.

Table [SEQ Table * ARABIC]: Increasing sample dilution to 50-fold

Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	2867	9.7	28.1	10247.4	102.5
2	4000	7009	18.5	11.5	3445.5	86.1
3	1600	13706	11.9	5.9	1809.7	113.1
4	640	26074	10.8	3.1	713.2	111.4
5	256	45257	12.4	1.8	186.4	72.8
6	102	53763	9.4	1.5	106.1	104.0
7	41	67269	11.5	1.2	45.1	109.9
8	0	80494	3.6	1.0		
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**		**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	11188	18.5		2234.1	94.7
HAMA M2	1900	13416	3.2		1852.8	97.5
HAMA M3	1270	23586	7.0		855.2	67.3
HAMA F1	853	27184	13.5		658.2	77.2
HAMA F2	1610	14766	12.7		1663.3	103.3
RF 818	504	29336	11.0		563.7	111.9
RF 822	366	34888	22.3		380.3	103.9
PCOS 1	1510	21846	3.1		972.1	64.4
PCOS 17	4640	7084	18.1		3411.7	73.5
Stanford M3 (10/15/12)	2000	17694	17.3		1326.4	66.3
Stanford F3 (10/15/12)	318	38945	7.0		286.7	90.2

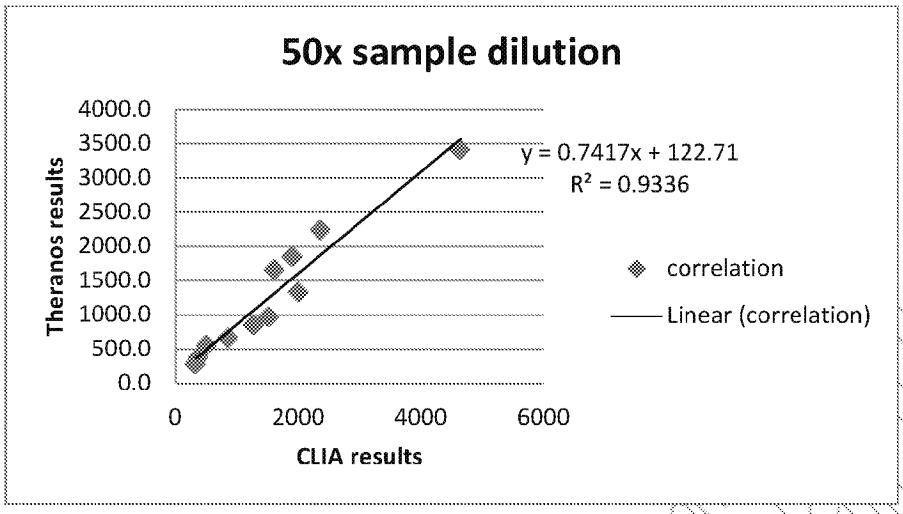


Figure [SEQ Figure * ARABIC]: Clinical sample correlation at 50x dilution

2.4.3 Re-visit of capture format

After promising result was obtained from increasing sample dilution to 50-fold, the capture format was re-visited to further improve clinical sample recovery. Both capture on tip and capture in solution were tested. To keep 50x sample dilution, Edison protocol Generic2_50x_competitive_10_10 was used.

More samples were analyzed with both methods and "capture in solution" showed better recovery although both methods had similar modulation and sensitivity. Bio Rad Lyphochek Immunoassay Controls were also re-analyzed in final condition to confirm the assay format. "Capture in solution" was chosen as final format.

Table [SEQ Table * ARABIC]: Calibration curve and samples with "Capture on tip" format at 50x sample dilution

Sample	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	2867	9.7	28.1	10247.4	102.5
2	4000	7009	18.5	11.5	3445.5	86.1
3	1600	13706	11.9	5.9	1809.7	113.1
4	640	26074	10.8	3.1	713.2	111.4
5	256	45257	12.4	1.8	186.4	72.8
6	102	53763	9.4	1.5	106.1	104.0
7	41	67269	11.5	1.2	45.1	109.9
8	0	80494	3.6	1.0		

Sample	CLIA Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
HAMA M1	2360	11188	18.5		2234.1	94.7
HAMA M2	1900	13416	3.2		1852.8	97.5
HAMA M3	1270	23586	7.0		855.2	67.3
HAMA F1	853	27184	13.5		658.2	77.2
HAMA F2	1610	14766	12.7		1663.3	103.3
RF 818	504	29336	11.0		563.7	111.9
RF 822	366	34888	22.3		380.3	103.9
PCOS 1	1510	21846	3.1		972.1	64.4
PCOS 17	4640	7084	18.1		3411.7	73.5
Stanford M3 (10/15/12)	2000	17694	17.3		1326.4	66.3
Stanford F3 (10/15/12)	318	38945	7.0		286.7	90.2
PCOS 2	1330	22018	6.7		959.8	72.2
PCOS 3	2160	11002	19.6		2270.9	105.1
PCOS 4	2200	20021	9.0		1113.2	50.6
PCOS 5	2670	13614	7.8		1823.2	68.3
Stanford M4 (10/15/12)	715	35392	28.7		367.1	51.3
Stanford M5 (10/15/12)	298	32571	3.3		447.7	150.3
Stanford F4 (10/15/12)	617	32606	10.0		446.6	72.4
Stanford F5 (10/15/12)	483	34631	16.8		387.2	80.2

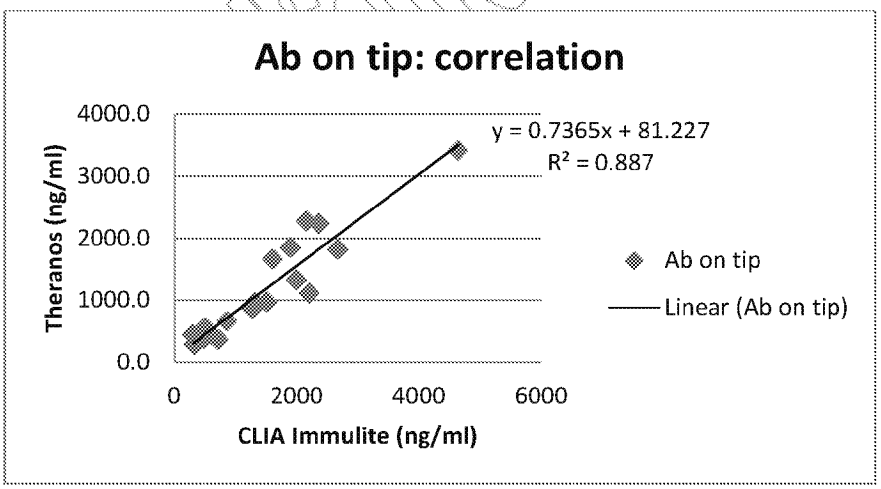


Figure [SEQ Figure * ARABIC]: Clinical sample correlation with "capture Ab on tip"

Table [SEQ Table * ARABIC]: Calibration curve and samples with "Capture in solution" format at 50x sample dilution

Sample	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. (ng/ml)	% accuracy
1	10000	2021	5.6	24.5	10107.1	101.1
2	4000	5082	18.5	9.8	3821.6	95.5
3	1600	10268	9.7	4.8	1536.3	96.0
4	640	15121	13.8	3.3	778.0	121.6
5	256	25971	8.7	1.9	217.2	84.8
6	102	33781	12.4	1.5	98.4	96.5
7	41	42513	14.7	1.2	44.2	107.8
8	0	49561	10.4	1.0	24.4	
Sample	**CLIA Conc. (ng/ml)**	**Mean RLU**	**%CV**	**Modulation**	**Back cal conc. (ng/ml)**	**% accuracy**
HAMA M1	2360	9119	9.2		1836.4	77.8
HAMA M2	1900	9554	8.9		1714.5	90.2
HAMA M3	1270	10537	21.7		1475.1	116.2
HAMA F1	853	14385	9.1		857.3	100.5
HAMA F2	1610	10295	3.2		1530.0	95.0
RF 818	504	18127	4.0		531.8	105.5
RF 822	366	20983	8.5		378.3	103.4
RF 827	<150	29541	14.4		149.7	
RF 828	<150	30545	6.4		135.2	
RF 829	<150	43941	11.7		39.1	
PCOS 1	1510	10262	6.0		1537.7	101.8
PCOS 17	4640	4028	2.5		4898.6	105.6
PCOS 2	1330	13494	1.5		966.9	72.7
PCOS 4	2200	9226	10.3		1805.3	82.1
PCOS 5	2670	7285	8.3		2494.3	93.4
Stanford M3 10/15/2012	2000	9952	13.2		1612.2	80.6
Stanford F3 10/15/2012	318	22083	3.9		333.4	104.8
Stanford M4 10/15/2012	715	14391	8.8		856.7	119.8
Stanford F4 10/15/2012	617	15599	8.7		731.0	118.5
Bio Rad 1	652	17456	13.4		577.7	88.6
Bio Rad 2	1300	12981	6.8		1037.6	79.8
Bio Rad 3	4310	4688	1.4		4172.6	96.8

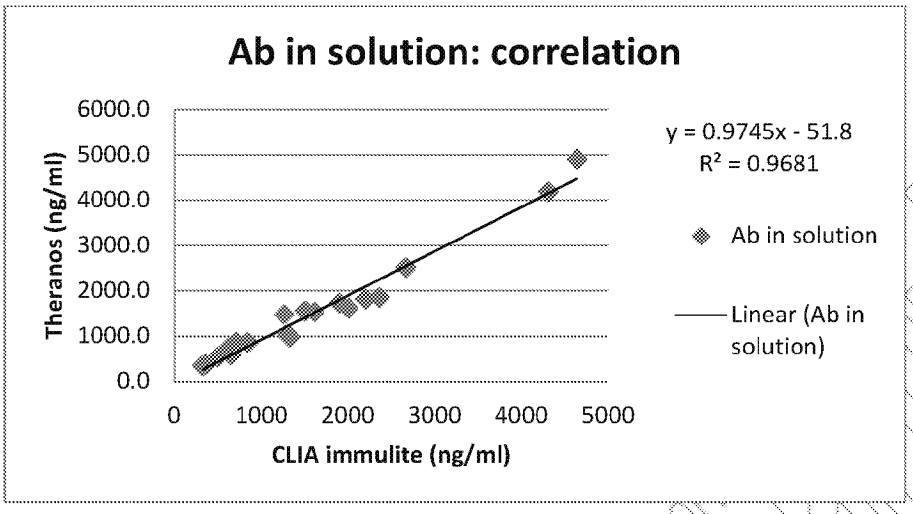


Figure [SEQ Figure * ARABIC]: Clinical sample correlation with "capture Ab in solution"

2.5 Verification and confirmation of final assay condition

2.5.1 Hematocrit effect and anticoagulant effect

Methods:

Whole blood, serum, EDTA plasma and heparin plasma samples from ten healthy donors (5 male and 5 female) were obtained in pairs. All samples were analyzed with final assay procedure. Hematocrit factor was calculated by comparing DHEAS results in whole blood and in EDTA plasma samples from the same donor. EDTA plasma, heparin plasma and serum from the same donor were also analyzed to compare the effect of anticoagulant.

Results:

Hematocrit factor was calculated to be 1.7 from the slope of plotting DHEAS results from EDTA plasma vs. results from whole blood.

The DHEAS results from EDTA plasma, heparin plasma and serum correlated with each other without showing significant different. This method could be used to analyze whole blood, EDTA plasma and heparin plasma.

Table [SEQ Table * ARABIC]: Results of matched matrix form healthy donors

	Whole blood			EDTA plasma			Heparin plasma			Serum		
Sample	Mean RLU	%CV	Cal. Conc. (ng/ml)	Mean RLU	%CV	Cal. Conc. (ng/ml)	Mean RLU	%CV	Cal. Conc. (ng/ml)	Mean RLU	%CV	Cal. Conc. (ng/ml)
M1	21438	7.8	456.1	16187	8.6	808.3	13870	10.6	1067.8	12459.1	10.3	1280.2

M2	14140	15.6	1032.6	9521	19.4	1948.7	8848	5.3	2169.0	9154.4	6.2	2064.5
M3	10753	15.4	1620.4	8576	14.2	2268.5	7249	7.9	2865.0	7326.0	5.9	2824.2
M4	14489	12.0	989.1	9392	2.3	1988.3	7958	9.3	2520.6	8242.7	11.8	2399.6
M5	10183	5.7	1761.6	6748	10.7	3153.1	6135	2.8	3570.5	5912.5	9.2	3743.1
F1	16176	0.2	809.4	11676	9.8	1422.9	10795	7.6	1610.5	11535.8	11.0	1450.7
F2	16456	15.4	783.6	11746	10.6	1409.3	10547	1.4	1669.4	11336.1	7.8	1491.6
F3	12866	10.6	1213.7	7783	1.5	2599.6	7880	22.8	2555.5	6517.7	3.7	3300.4
F4	10869	13.3	1593.5	7314	9.1	2830.6	6739	8.4	3158.3	6982.0	10.0	3013.2
F5	19445	18.1	562.7	17078	11.6	730.0	16585	5.3	772.1	14944.9	12.3	936.0

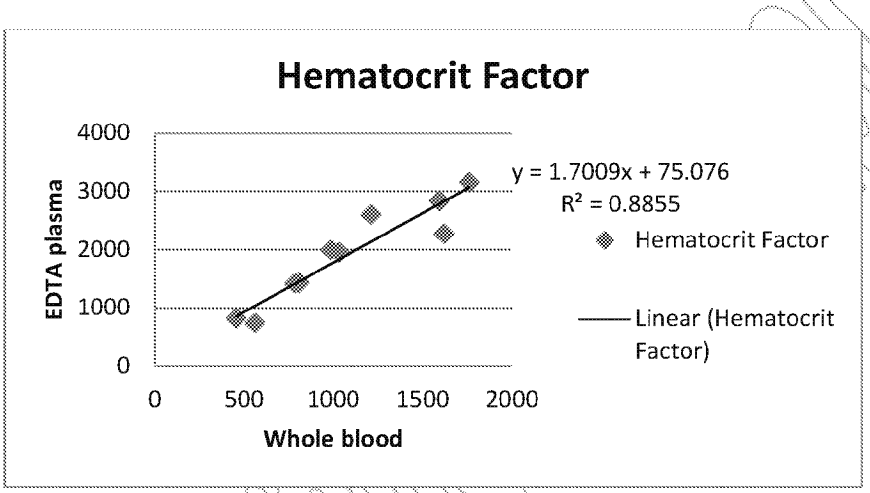


Figure [SEQ Figure * ARABIC]: Hematocrit factor

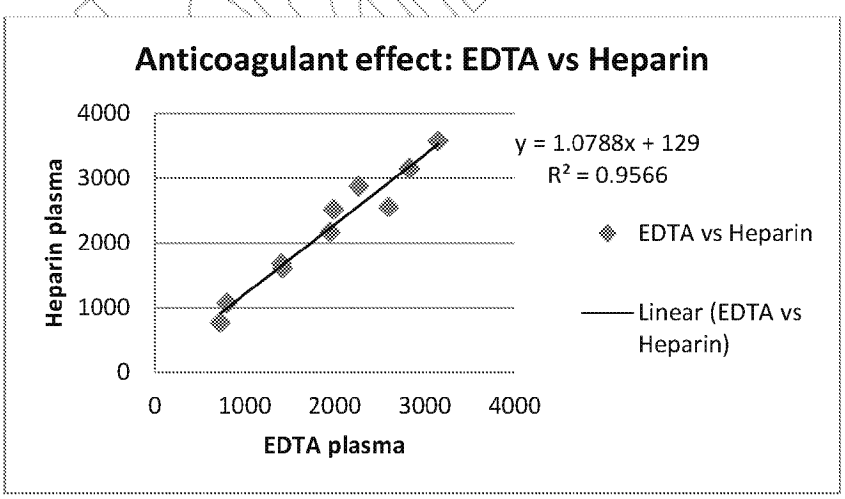


Figure [SEQ Figure * ARABIC]: Anti-coagulant effect

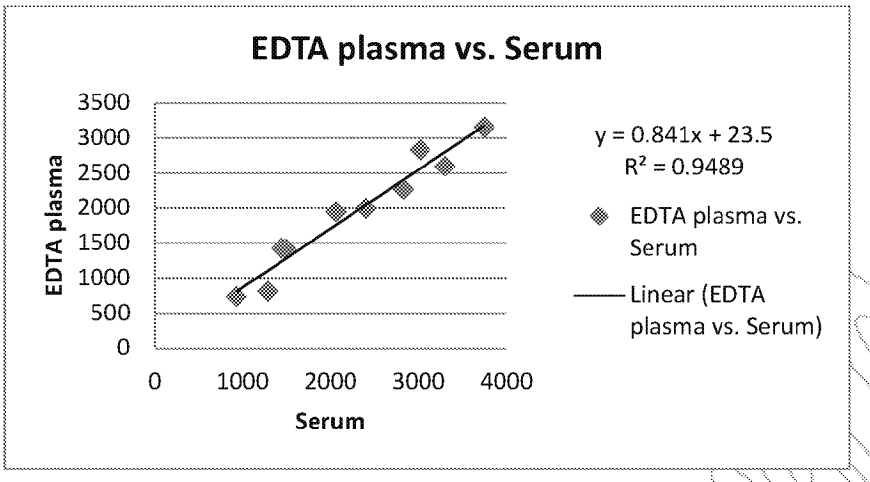


Figure [SEQ Figure * ARABIC]: Results of EDTA plasma vs. serum

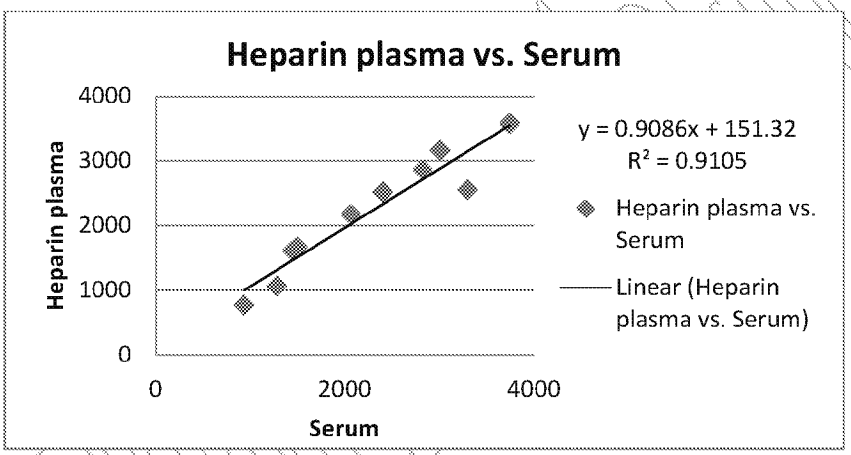


Figure [SEQ Figure * ARABIC]: Results of heparin plasma vs. serum

2.5.2 Effect of positive HAMA and RF factor

Five HAMA positive serum samples and five RF positive samples from PromedDx were analyzed with final assay condition. All samples tested showed results agreeing with reference method. The results indicated that HAMA positive and RF positive status didn't affect DHEAS analysis in Theranos method.

Table [SEQ Table * ARABIC]: Results of analysis of HAMA positive samples and RF positive samples

Sample ID	CLIA Conc. (ng/ml)	Mean RLU	%CV	Back cal	% accuracy
HAMA M1	2360	9119	9.2	1836.4	77.8
HAMA M2	1900	9554	8.9	1714.5	90.2

HAMA M3	1270	10537	21.7	1475.1	116.2
HAMA F1	853	14385	9.1	857.3	100.5
HAMA F2	1610	10295	3.2	1530.0	95.0
RF 818	504	18127	4.0	531.8	105.5
RF 822	366	20983	8.5	378.3	103.4
RF 827	<150	29541	14.4	149.7	
RF 828	<150	30545	6.4	135.2	
RF 829	<150	43941	11.7	39.1	

2.5.3 Effect of interfering matrixes

Hemolyzed serum, lipemic serum, and icteric serum were tested to evaluate potential interference. All samples had acceptable recovery comparing to results from reference method. Hemolyzed serum, lipemic serum, and icteric serum didn't show significant matrix effect to interfere DHEAS measurement in this assay.

Table [SEQ Table * ARABIC]: Results of evaluating interfering matrixes

Sample ID	CLIA Conc. (ng/ml)	Mean RLU	%CV	Back cal conc. (ng/ml)	% accuracy
Hemolyzed 1	836	13342	5.5	987.3	118.1
Hemolyzed 2	1640	10720	14.7	1435.4	87.5
Hemolyzed 3	2090	8184	24.6	2138.4	102.3
Hemolyzed 5	364	21473	9.0	357.5	98.2
Hemolyzed 6	631	17979	6.8	541.5	85.8
Icteric 1	3170	6099	11.3	3104.3	102.3
Icteric 8	855	17256	9.0	592.2	98.2
Icteric 17	199	24647	18.4	250.5	85.8
Icteric 18	3780	7155	5.6	2552.6	67.5
Icteric 19	675	16372	6.7	662.0	98.1
Lipemic 683	1410	10663	20.8	1447.5	102.7
Lipemic 685	2270	10103	10.7	1575.2	69.4
Lipemic 687	2140	10317	20.6	1524.9	71.3
Lipemic 688	6140	3519	5.8	5632.6	102.3
Lipemic 689	786	19883	17.9	430.4	98.2

2.6 Assay final evaluation and analysis of clinical samples

2.6.1 Calibrator verification

DHEAS stock material was purchased from Cerilliant and Sigma. Two sets of calibrators made from both sources were sent to CLIA lab for analysis on reference method.

Both two sets of calibrators were confirmed by reference method. As Cerilliant material is certified standard material, it was used to prepare calibrators in final assay evaluation.

Table [SEQ Table * ARABIC]: Calibrator verification

DHEAS source		**Cerilliant**	**Sigma**
Calibrator	**Nominal conc. (ug/dL)**	**Conc. from CLIA (ug/dL)**	**Conc. from CLIA (ug/dL)**
Calibrator 1	1000	997	907
Calibrator 2	500	492	394
Calibrator 3	250	235	204
Calibrator 4	100	88.8	79.3
Calibrator 5	50	42.2	44.3
Calibrator 6	25	20.7	24.9
Calibrator 7	10	< 15.0	< 15.0
Calibrator 8	0	< 15.0	< 15.0

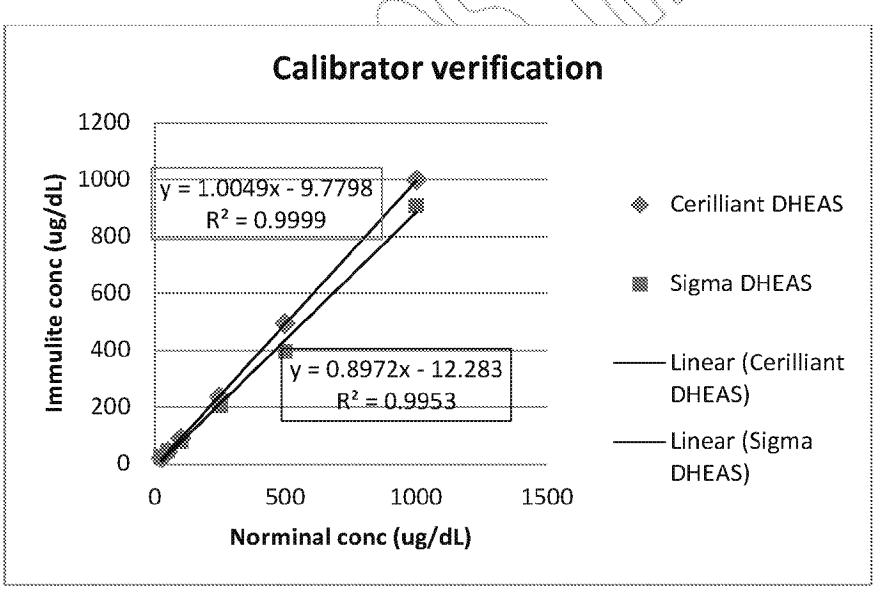


Figure [SEQ Figure * ARABIC]: Calibrator verification

2.6.2 Evaluation of assay LLOQ and ULOQ

Calibration curve was generated with final protocol Generic2_50x_competitive_10_10 and analyzed using Dexter software. LLOQ of this assay was achieved at 41ng/ml and ULOQ was at 10000ng/ml with acceptable precision and accuracy.

Table [SEQ Table * ARABIC]: Calibration curve for Dexter analysis

Calibrator	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Back cal conc. From Dexter (ng/ml)	% accuracy
1	10000	2483	9.5	24.8	10207.3	102.1
2	4000	4877	7.7	12.6	4755.5	118.9
3	1600	13099	13.2	4.7	1177.5	73.6
4	640	18912	11.8	3.3	596.0	93.1
5	256	27307	14.5	2.3	253.1	98.9
6	102	36114	9.4	1.7	107.0	104.9
7	41	47319	3.7	1.3	32.8	80.1
8	0	61549	9.7	1.0	OORL	--

Table [SEQ Table * ARABIC]: Dexter parameters

Model Type	LogLin 4PL
Model Equation	log10(RLU) = b1+(b2-b1)/(1+(Conc/b3)^b4
Calibration Equation	Conc.=70670.564*(((4.883-0.162)/(log10(RLU)-0.162))-1)^(1/0.401)
b1	0.162
b2	4.883
b3	70670.564
b4	0.401
LLOQ	41ng/ml
ULOQ	10,000ng/ml
LLOQ accuracy	81%
LLOQ precision	19.2%
ULOQ accuracy	103%
ULOQ precision	9.7%

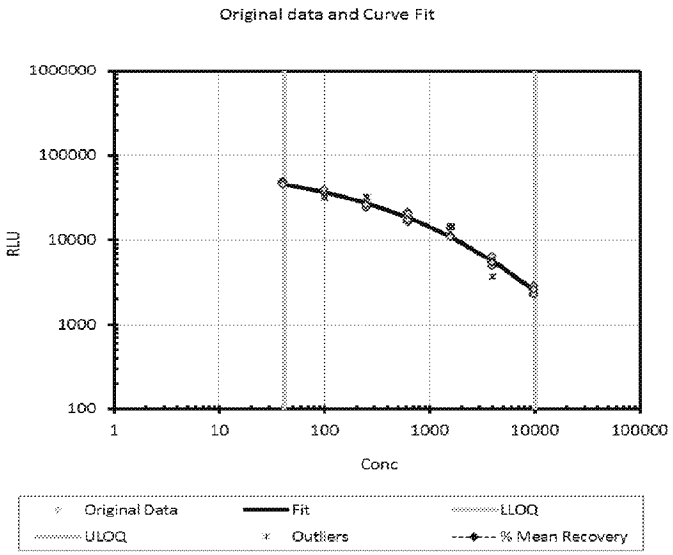


Figure [SEQ Figure * ARABIC]: Calibration curve analysis by Dexter

2.6.3 Analysis of clinical samples

2.6.3.1 Calibration curve after fine tune assay conditions

A large batch tips were coated and a fine tune of assay condition was to set capture antibody concentration to 1:5000 dilution and detection conjugate concentration at 12.5ng/ml. A calibration curve was generated for analyzing clinical samples.

Table [SEQ Table * ARABIC]: Calibration curve for clinical sample analysis

Sample	Conc. (ng/ml)	Mean RLU	%CV	Modulation	Mean Conc. cal from Dexter (ng/ml)	% accuracy
1	10000	2069	23.2	23.4	10003.4	100.0
2	4000	3789	18.1	12.8	4432.1	110.8
3	1600	9278	14.4	5.2	1405.0	87.8
4	640	13972	6.9	3.5	764.7	119.5
5	256	20058	9.3	2.4	281.4	109.9
6	102	27127	10.2	1.8	100.3	98.3
7	41	34327	6.3	1.4	53.4	130.3
8	0	48480	2.1	1.0	OORL	--
Calibration Equation		conc. = 20363.610*(((4.69-1.363)/(log10(RLU)-1.363))-1)^(1/0.509)				

2.6.3.2 Analysis of clinical samples

Total 40 samples (Twenty EDTA plasma samples from healthy donors, seventeen serum samples from polycystic ovarian syndrome (PCOS) patients, and three serum samples from pediatric patients) were analyzed using the final assay protocol. Correlation was calculated by plotting DHEAS results from this Theranos assay vs. results from reference assay.

The results of total 40 samples agreed with reference method very well.

Table [SEQ Table * ARABIC]: Summary of clinical samples

Sample ID	DHEAS conc from CLIA lab (ng/ml)	Mean RLU	%CV	Theranos cal conc. (ng/ml)	% Accuracy
Stanford 05252012 M1	895	14522	4.4	768.5	85.9
Stanford 05252012 M2	1600	10911	7.5	1272.3	79.5
Stanford 05252012 M3	768	14990	9.3	722.7	94.1
Stanford 05252012 M4	449	19558	17.0	405.0	90.2
Stanford 05252012 M5	1630	10854	11.9	1283.3	78.7
Stanford 05252012 M6	2340	7001	5.1	2450.9	104.7
Stanford 05252012 M7	1290	12536	11.8	1006.5	78.0
Stanford 05252012 M8	788	17250	10.2	540.6	68.6
Stanford 05252012 M9	282	16965	12.0	560.5	198.8
Stanford 05252012 M10	1360	12255	12.3	1047.0	77.0
Stanford 05252012 F1	1630	9614	12.1	1553.8	95.3
Stanford 05252012 F2	1930	8169	6.7	1977.4	102.5
Stanford 05252012 F3	531	15010	15.0	720.7	135.7
Stanford 05252012 F4	1760	9947	14.9	1474.0	83.7
Stanford 05252012 F5	780	15445	14.5	681.0	87.3
Stanford 05252012 F6	2180	6159	5.0	2904.4	133.2
Stanford 05252012 F7	1610	9616	12.9	1553.1	96.5
Stanford 05252012 F8	813	14300	11.4	791.5	97.4
Stanford 05252012 F9	1300	11044	8.2	1247.4	96.0
Stanford 05252012 F10	1900	9073	6.1	1696.1	89.3
PCOS 1	1510	10528	10.8	1347.9	89.3
PCOS 2	1330	10961	5.1	1262.9	95.0
PCOS 3	2160	6754	9.2	2572.2	119.1
PCOS 4	2200	8608	13.1	1833.0	83.3
PCOS 5	2670	7000	11.9	2451.1	91.8
PCOS 6	1910	6673	9.0	2613.8	136.8
PCOS 7	663	14196	8.2	802.5	121.0
PCOS 8	1420	9287	8.1	1637.6	115.3

PCOS 9	1980	7960	0.2	2051.6	103.6
PCOS 10	3120	5804	8.1	3135.5	100.5
PCOS 11	1830	8347	34.1	1916.8	104.7
PCOS 12	2000	7602	16.5	2188.7	109.4
PCOS 13	2000	8260	12.5	1946.0	97.3
PCOS 14	961	11031	8.0	1249.7	130.0
PCOS 15	1200	10325	6.3	1390.3	115.9
PCOS 16	1510	10036	28.1	1453.8	96.3
PCOS 17	4640	3896	12.7	5084.5	109.6
Peditrics 16	359	22443	1.6	282.9	78.8
Peditrics 17	359	18443	16.7	465.4	129.6
Peditrics 15	<150	33324	26.5	63.3	--

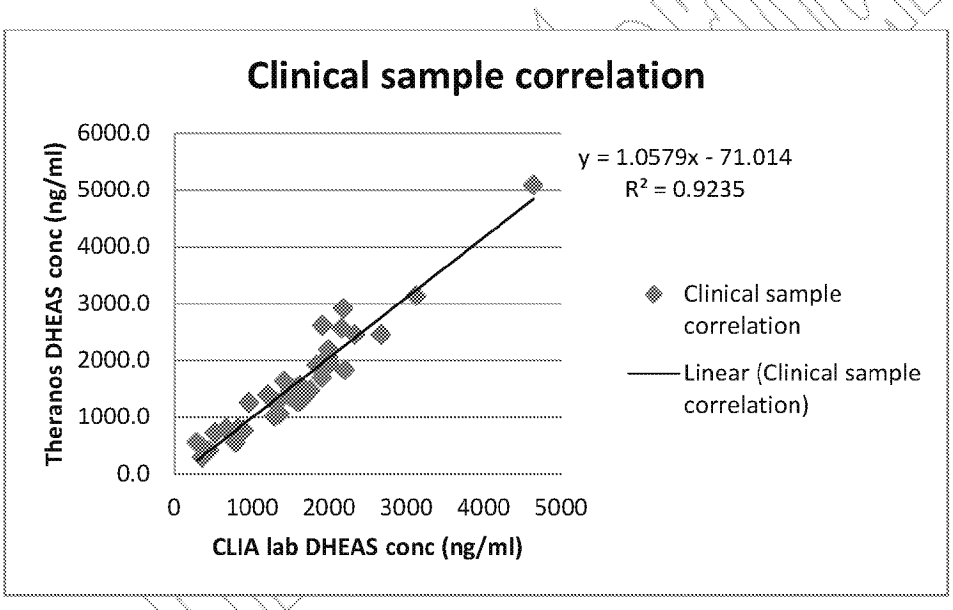


Figure [SEQ Figure * ARABIC]: Correlation of clinical samples to reference method

2.7 Stability

Assay stability monitoring is on-going with reagents and coated tips stored at 4°C.

3 REFERENCES

P.D. Krobath, et al. DHEA and DHEA-S: A review, J Clin Pharmacol 39: 327-48, 1999

N. Tagawa, et al. Serum Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, and Pregnenolone Sulfate Concentrations in Patients with Hyperthyroidism and Hypothyroidism, Clinical Chemistry 46(4): 523–528, 2000

S. Benton, et al. Measured Dehydroepiandrosterone Sulfate Positively Influences Testosterone Measurement in Unextracted Female Serum: Comparison of 2 Immunoassays with Testosterone Measured by LC-MS, Clinical Chemistry 57(7): 1074–1083, 2011