



Follicle-Stimulating Hormone Assay Validation Report

Theranos, Inc.

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1. Analyte Background

Follicle-stimulating hormone (FSH) is a hormone synthesized and secreted by gonadotropes in the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the human body. Analyte concentration reference ranges from the literature:

FEMALES:	mIU/mL
Follicular phase	3.5 - 12.5
Ovulation phase	4.7 - 21.5
Luteal phase	1.7 - 7.7
Postmenopause	25.8 - 134.8
MALES:	1.5 - 12.4

2. Assay Specifications

The Therasnos assay for FSH is a sandwich ELISA usable with human whole blood, plasma and serum. The reportable range for the assay is 200-3 mIU/mL.

3. Reference Assays and Standards

The following commercial ELISA kit has been used in house as a reference assay:

Genway FSH-Elisa Kit Catalog# 40-052-115017
Reported range: 200-5 mIU/mL

Standardization: Therasnos FSH standards are calibrated against the World Health Organization's First International Standard for Follicle Stimulating Hormone, Recombinant Human FSH (Code 92/642).

4. Cross Reactivity

The FSH assay was tested for cross reactivity with Luteinizing Hormone (LH) and human Chorionic Gonadotropin (hCG). Cross reactivity was determined by testing the analytes mentioned below with the finalized antibody pair.

Result: No significant cross reactivity was observed.

Test Substance	Test Substance Level	% Cross Reactivity
LH	20 ng/mL	0.83
hCG	216 ng/mL	0.06

5. Interfering Substances

The FSH assay was tested for interference to Luteinizing Hormone and human Chorionic Gonadotropin . The test substances were added to FSH calibrators in assay buffer at levels higher than the expected clinical concentrations of these test substances, and the impact on recovery of FSH was measured.

Result: No significant interference was observed, recovery of FSH was within 10 % of nominal levels in the mid range of the assay.

Test Substance	Test Substance Level	Nominal [FSH] mIU/mL	Recovered [FSH] mIU/mL	% Recovery
LH	60 ng/mL	40.0	44.0	110
hCG	648 ng/mL	40.0	43.5	109

6. Precision

Inter-Reader Precision

Inter-reader Precision test was evaluated by running a single analyte level (2 mIU/mL) on 24 instruments.

Result: Total Inter-Reader concentration CV % at 2 mIU/mL was 14%

Cartridge #	Recovered Conc. mIU/mL
1	2.1
2	1.6
3	2.1
4	2.4
5	2.1
6	2.3
7	1.6
8	1.9
9	2.0
10	2.6
11	2.0
12	2.1

Cartridge #	Recovered Conc. mIU/mL
13	2.1
14	2.3
15	1.9
16	2.6
17	2.0
18	1.8
19	2.1
20	2.0
21	1.9
22	2.1
23	2.6
24	2.7

Nominal [FSH] mIU/mL	Recovered [FSH] mIU/mL			
	Mean Conc.	StDev	CV %	% Recovery
2.0	2.1	0.30	14	106

Inter-Cartridge Lot Precision

Precision of the assay across multiple reagent lots was evaluated by running a 6 point standard curve on three different reagent lots over multiple instruments, 3 cartridges per point.

The Average inter-lot concentration CV was 7.7 %.

FSH: Concentration CV %

[FSH]	Cartridge	Conc mIU/mL			Total Concentration CVs			Average% Recovery
		Lot 1	Lot 2	Lot 3	Mean	StDev	CV %	
200	1	197.6	200.9	200.2	199.5	1.3	0.6	100
	2	200.7	198.8	199.4				
	3	201.2	198.2	198.6				
40	1	44.0	43.1	37.4	40.3	3.9	9.7	101
	2	42.2	43.6	32.1				
	3	42.2	40.7	37.5				
8	1	8.4	7.6	6.9	7.9	0.9	11.4	98
	2	9.3	7.2	7.0				
	3	8.9	7.1	8.4				
2	1	2.1	1.9	2.2	2.0	0.2	7.8	102
	2	1.7	2.0	2.0				
	3	2.1	2.0	2.3				
0.5	1	0.5	0.5	0.5	0.5	0.0	5.0	100
	2	0.5	0.5	0.5				
	3	0.5	0.5	0.5				
0	1	0.3	0.3	0.3	0.3	0.0	3.5	-
	2	0.3	0.3	0.3				
	3	0.3	0.3	0.3				

7. Control Comparison

a) On the Genway ELISA kit

The commercially available analyte used for the Theranos assay development was tested on the Genway Elisa Kit Catalog # 40-052-1150171.

Result: Recovery was within an acceptable range.

The commercially available analyte used by the Theranos assay was tested on the Genway FSH ELISA kit

Nominal [FSH] mIU/mL	Recovered [FSH] mIU/mL			
	Mean Conc	StDev	CV%	% Recovery
196	221.3	12	5	113
98	92.6	10	11	94
32	38.3	0	1	120
16	18.3	0	1	114
8.0	8.4	1	9	107
4	4.1	0	3	104
2	OORL	-	-	-
1	OORL	-	-	-
0.5	OORL	-	-	-
0	OORL	-	-	-

OORL: Out of Range Low

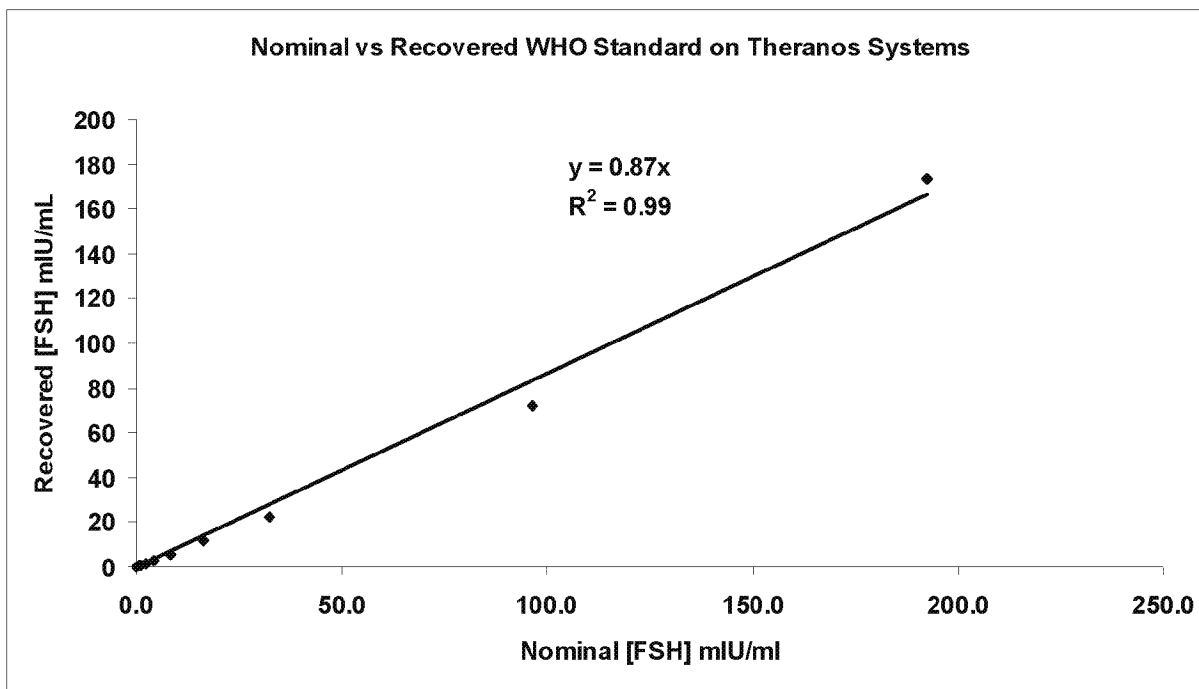
b) WHO Standard Recovery

Recovery of the NISBC WHO FSH standard (NIBSC 92/642) on the Theranos System was tested.

Result: Average % recovery was 87 % of nominal. Therefore the Theranos System was subsequently recalibrated to the WHO standard.

WHO Standard curve in Assay Buffer

[FSH] mIU/mL	Signal (RLU)			Recovered [FSH] mIU/mL			
	Mean RLU	StDev	% CV	Mean Conc.	StDev	CV%	% Recovery
192	139340	9045	6	174	9	5	90
96	52331	2796	5	72	4	5	75
32	18371	425	2	23	1	3	70
16	10974	960	9	12	1	11	75
8	5931	74	1	6	0.1	2	69
4	3773	228	6	3	0.2	8	78
2	1737	124	7	1.2	0.1	9	60
1	1131	84	7	0.7	0.1	9	72
0.5	788	59	7	0.5	0.0	8	96
0	451	58	13	0.3	0.0	13	

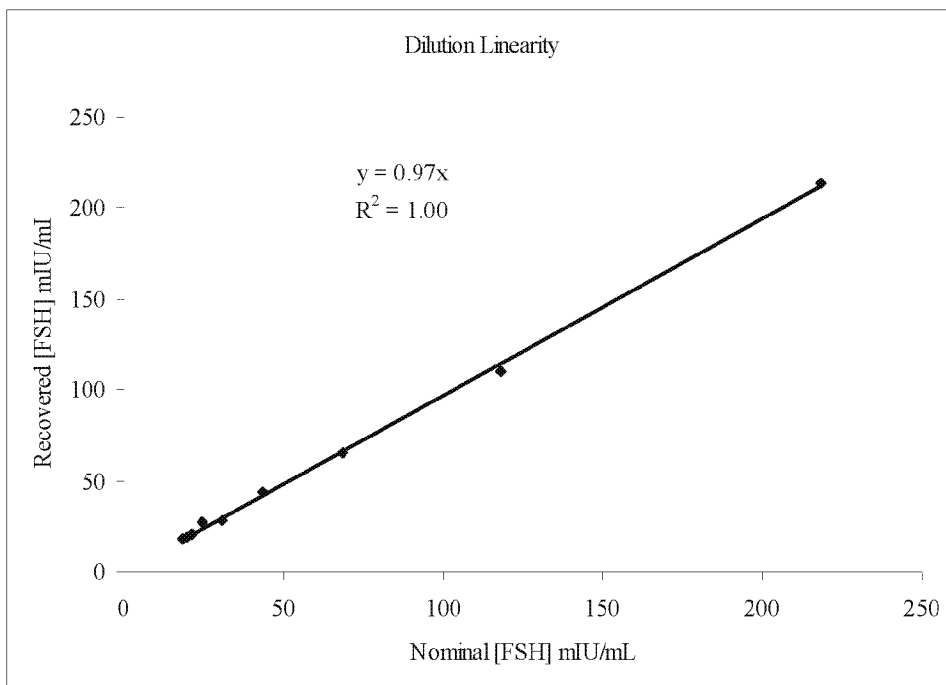


8. Dilution Linearity

A sample with a low endogenous serum FSH level was spiked with 200 mIU/mL FSH and recombined with the low FSH starting sample to determine dilution linearity. Calibration was performed using an assay buffer standard curve.

Result: The % recovery ranged from 85-110%.

Nominal [FSH] mIU/mL	Signal (RLU)			Recovered [FSH] mIU/mL			
	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
218	292013	29468	10	240	35	15	110
118	159516	8802	6	104	7	7	88
68	97912	2200	2	59	1	2	87
43	67091	7264	11	39	4	11	92
30	43113	1625	4	26	1	4	85
24	41943	2208	5	25	1	5	104
21	32116	1124	4	20	1	3	94
19	30275	1283	4	19	1	4	96
17.7	28793	846	3	18	0	3	100



9. Whole Blood Spike Recovery

Whole blood samples were spiked with FSH at different levels across the assay range then analyzed on the Theranos System, calibrated on assay buffer calibrators. The nominal values for FSH were computed by measuring the endogenous level and adding it to the spike level. Recovery was excellent for all samples.

Whole Blood Sample 1

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	193.3	121365	8199	7	175.7	22	13	91
96	97.3	85217	4275	5	96.1	8	8	99
32	33.3	32078	4375	14	26.9	4	15	81
16	17.3	17334	774	4	14.4	1	4	84
8	9.3	9121	899	10	7.9	1	9	85
0	1.3	1891	138	7	1.3	0	11	

Whole Blood Sample 2

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	193.0	128529	7126	6	195.2	20	10	102
96	97.0	87216	9623	11	100.3	18	18	104
32	33.0	33875	1900	6	28.5	2	6	89
16	17.0	17324	812	5	14.4	1	4	90
8	9.0	9464	622	7	8.2	1	6	102
0	1.0	1680	67	4	1.0	0	6	

Whole Blood Sample 3

[FSH] mIU/mL		Signal (RLU)			Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean RLU	StDev	CV %	Mean Conc.	StDev	CV %	% Recovery
192	192.5	124989	10090	8	185.8	27	15	97
96	96.5	93644	5614	6	111.9	11	10	117
32	32.5	37191	3648	10	31.7	3	11	99
16	16.5	19091	466	2	15.8	0.3	2	99
8	8.5	8619	465	5	7.5	0.4	5	94
0	0.5	1151	84	7	0.5	0.0	14	

10. Matrix Effects

The impact of various sample matrixes was evaluated on the assay by spiking in FSH across the range of the assay into commercially obtained hemolyzed, lipemic and icteric sera. Recovery of spiked samples was calculated on an assay buffer standard curve.

Result: Recovery was within the acceptable range for all tested matrixes.

Spiked into Hemolyzed Serum

[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	163.9	163.6	2	1	100
80.0	83.9	86.7	6	7	103
40.0	43.9	44.8	3	6	102
20.0	23.9	25.8	1	5	108
10.0	13.9	12.1	0	4	87
0.0	3.9	3.9	0	9	100

Spiked into Lipemic Serum

[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	164.5	160.9	6	4	98
80.0	84.5	95.1	11	11	113
40.0	44.5	47.5	6	12	107
20.0	24.5	25.5	2	9	104
10.0	14.5	17.9	2	13	124
0.0	4.5	4.5	1	15	-

Spiked into Icteric Serum

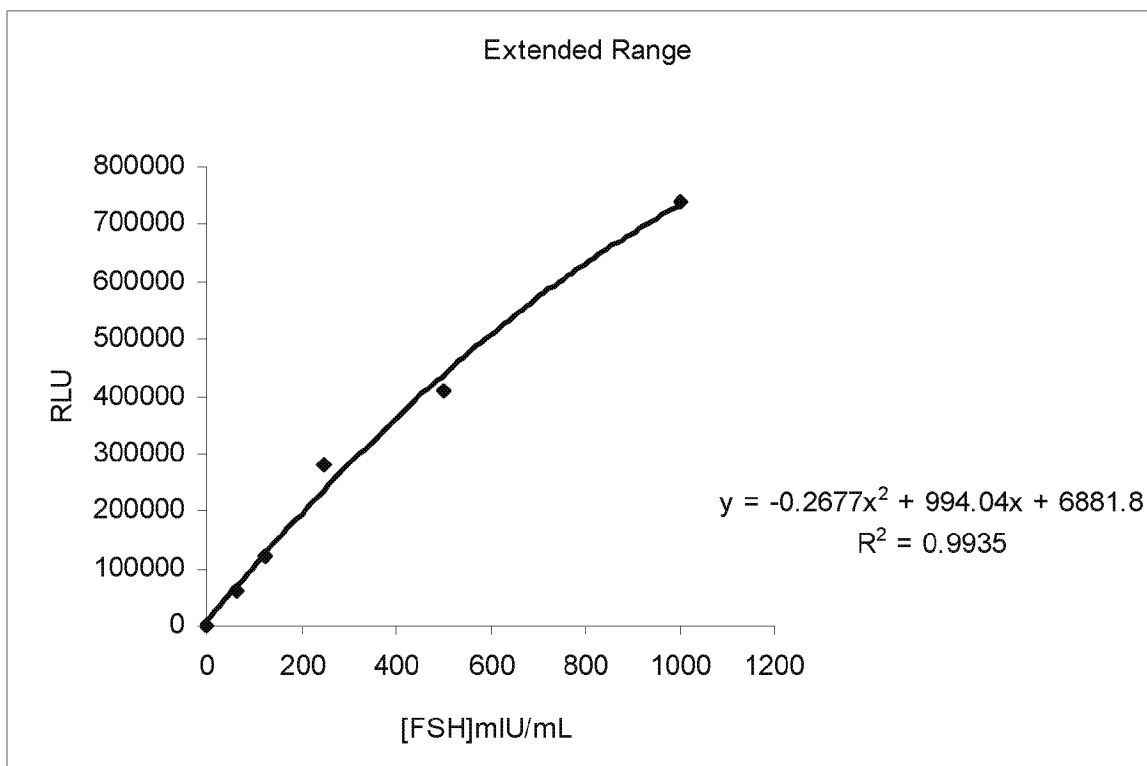
[FSH] mIU/mL		Recovered [FSH] mIU/mL			
Spiked	Nominal	Mean Conc.	StDev	CV %	% Recovery
160.0	163.5	154.3	7	5	94
80.0	83.5	83.2	4	5	100
40.0	43.5	53.4	2	5	123
20.0	23.5	20.0	2	8	85
10.0	13.5	16.5	1	9	122
0.0	3.5	3.5	0	14	-

11. Extended Range

A standard curve with levels up to five times the normal range was run to check for a high dose hook effect.

Result: No hook effect was observed.

[FSH]mIU/mL	Mean RLU	StDev	CV %
1000	737502	60311	8
500	410291	8450	2
250	278857	4201	2
125	123189	3070	2
63	60790	3771	6
0	547	27	5



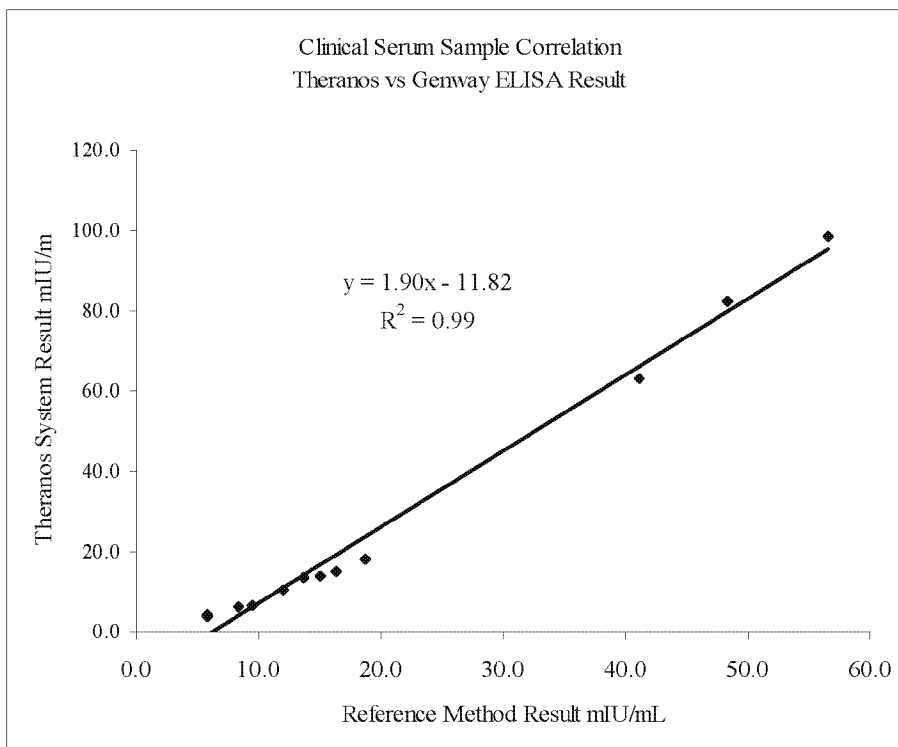
12. Validation in Clinical Samples

FSH assay was validated by testing 18 clinical samples from different stages of the menstrual cycle, pregnancy and postmenopausal patients on the Theranos System and on the Genway FSH ELISA (Cat #40-0521150171).

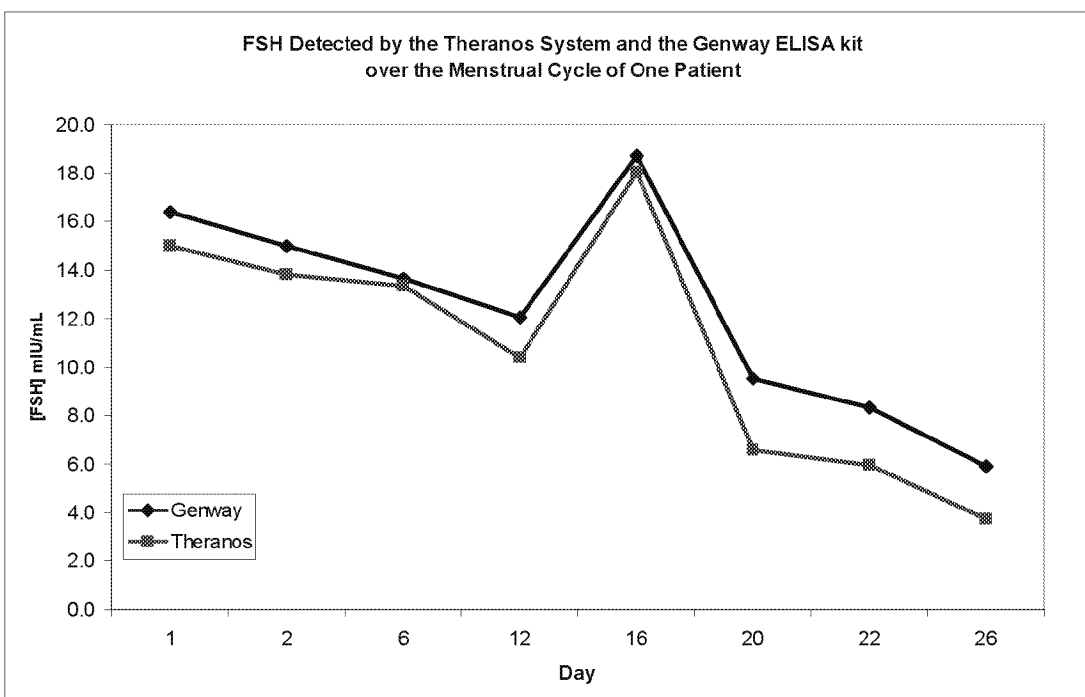
Result: Correlation of the Theranos result to Genway result was $R^2=0.99$.

Sample ID	Sample Type	Genway Mean [FSH] mIU/mL	Theranos System [FSH] mIU/mL		
			Mean Conc.	Std.Dev.	CV %
1	Menstrual	16.4	15.0	2.5	16
2	Menstrual	15.0	13.8	1.2	9
6	Menstrual	13.6	13.3	1.6	12
12	Menstrual	12.0	10.4	1.5	14
16	Menstrual	18.7	18.1	2.4	13
20	Menstrual	9.5	6.6	1.1	16
22	Menstrual	8.3	6.0	0.6	11
26	Menstrual	5.9	3.7	0.2	5
50	Pregnancy	OORL	OORL	-	-
51	Pregnancy	OORL	OORL	-	-
53	Pregnancy	1.6	OORL	-	-
54	Pregnancy	OORL	OORL	-	-
56	Pregnancy	5.8	4.2	0.8	20
62	Pregnancy	6.9	OORL	-	-
63	Pregnancy	10.6	OORL	-	-
80	Post-Menopausal	41.1	63.1	6.5	10
81	Post-Menopausal	56.6	98.4	6.6	7
82	Post-Menopausal	48.3	82.4	6.5	8

OORL: Out of Range Low



For a congruent set of samples from a menstrual cycle, we compared results for the Genway and Theranos systems. There was excellent agreement of both the absolute values and of the trends in analyte concentration over time.



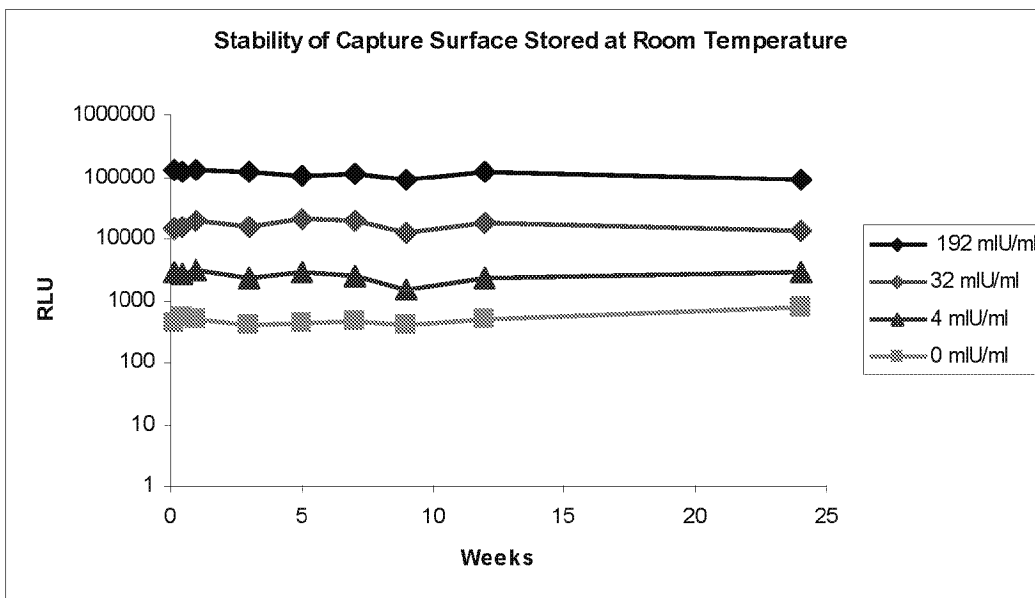
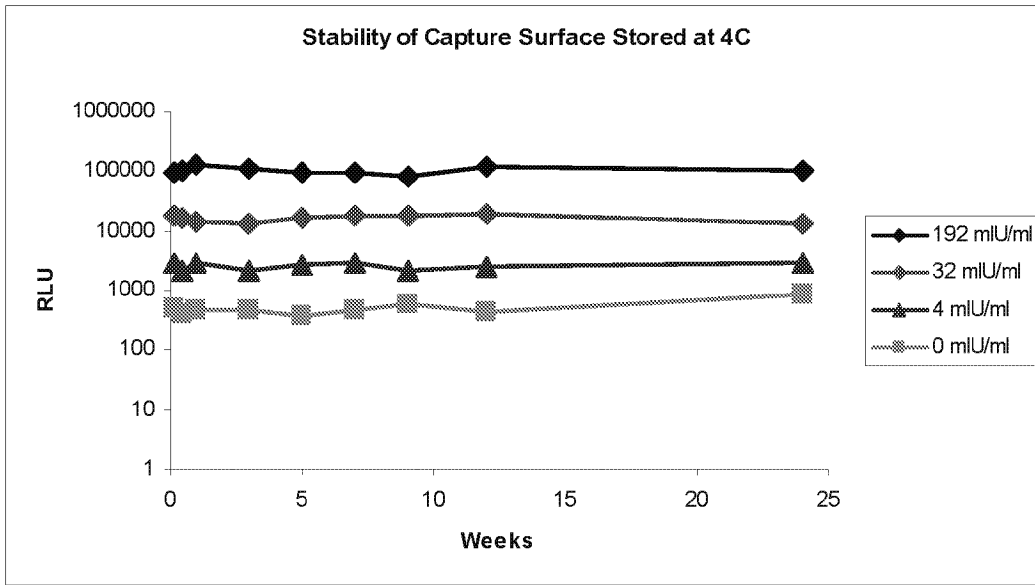
13. Stability

For each stability time point, all reagents are formulated fresh except the test reagent. In addition, a control is included with all reagents formulated fresh from stock materials.

Result: The capture surface and detection antibody show good stability up to 24 weeks.

Capture Surface Stability

Time point	FSH mIU/mL	Control		Stored at 4°C		Stored at Room Temp.	
		Mean RLU	CV %	Mean RLU	CV %	Mean RLU	CV %
Day 1	192	89334	8	92443	13	131813	5
	32	15078	7	17729	6	14228	11
	4	1626	10	2821	4	2944	3
	0	248	9	494	7	431	7
Week 1	192	119356	10	125498	7	124741	8
	32	13787	14	14762	16	19686	18
	4	2358	9	2827	3	3060	10
	0	352	1	479	6	495	10
Week 3	192	93480	5	109203	19	116024	11
	32	13596	11	13037	11	15419	33
	4	1862	10	2158	11	2371	15
	0	277	5	456	9	403	10
Week 5	192	100002	11	95136	11	101719	11
	32	17714	15	16252	8	21310	19
	4	3067	6	2615	14	2978	5
	0	456	9	379	7	417	9
Week 7	192	97913	12	94121	12	108133	20
	32	18719	7	17873	19	19233	26
	4	2642	22	2991	10	2468	19
	0	468	19	468	7	471	5
Week 9	192	75819	20	84401	1	90826	4
	32	12586	11	17242	8	12079	6
	4	2476	17	2212	21	1547	5
	0	549	3	578	10	414	5
Week 12	192	124197	10	116690	9	118224	9
	32	20272	22	19697	16	18190	24
	4	2998	6	2542	11	2333	10
	0	501	8	446	7	483	6
Week 24	192	90320	8	101466	5	91124	10
	32	15935	10	13443	8	13553	21
	4	2541	9	2937	9	2834	6
	0	1096	2	889	11	750	6



Detection antibody stability was monitored on a microtitre plate.

Detection Antibody Stability

Time point	[FSH] mIU/mL	Control		Stored at 4°C		Stored at Room Temp.	
		Mean RLU	CV %	Mean RLU	CV %	Mean RLU	CV %
Day 1	200	19675	1	20323	4	20019	4
	8	977	10	826	4	934	8
	0.5	242	9	228	6	329	25
	0	225	11	183	11	208	7
Week 1	200	17256	2	18154	4	17626	5
	8	932	7	718	8	1156	13
	0.5	241	6	229	10	226	14
	0	147	9	193	1	178	4
Week 3	200	18578	2	18674	1	18406	3
	8	831	2	947	4	963	10
	0.5	355	14	336	3	377	5
	0	276	8	248	16	251	14
Week 5	200	18605	1	19202	8	19324	4
	8	914	3	1343	4	1301	7
	0.5	383	2	398	9	399	4
	0	349	2	318	5	300	15
Week 7	200	20689	11	20606	7	20724	2
	8	936	14	1007	4	759	13
	0.5	328	10	273	30	324	11
	0	187	7	211	12	228	21
Week 9	200	19460	4	19055	3	18829	1
	8	1000	7	1014	7	1029	7
	0.5	345	30	369	15	400	10
	0	247	13	276	11	251	24
Week 12	200	18981	5	18766	3	18794	3
	8	1215	10	1157	14	983	8
	0.5	377	14	304	14	277	11
	0	274	23	220	12	260	8
Week 24	200	23775	2	21881	4	23716	1
	8	1242	7	1178	6	1212	7
	0.5	331	16	306	12	343	3
	0	250	11	264	6	321	11

