Message

From: Gary Frenzel [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=GFRENZEL]
Sent: 12/9/2009 7:25:52 PM
To: 'constance.cullen@spcorp.com' [constance.cullen@spcorp.com]
Subject: FW: Validation Report

Hi Connie, I just wanted to make sure that you received this report. We are looking forward to discussing it with you. Gary

Gary Frenzel
VP Assay Systems
Theranos
3200 Hillview Ave,
Palo Alto, Ca 94304

From: Gary Frenzel
Sent: Thursday, December 03, 2009 2:29 PM
To: 'constance.cullen@spcorp.com'
Subject: Validation Report

Hi Connie, I was asked to send this report on to you, and if you can forward to the proper people. After you and your group have an opportunity to go through it, let us know if you would like to arrange a phone conference to discuss the results. Thanks Gary

Gary Frenzel
VP Assay Systems
Theranos
3200 Hillview Ave,
Palo Alto, Ca 94304

theranos
redefining healthcare

Theranos Multiplexed Assay Panel Validation Report

Human IL-6, Human TNF-α, Human CRP (hs)

Contents

1. Introduction

The Theranos Assay System is a fully automated means for measuring concentrations of analytes (biomarkers, drugs) using immunoassay methodology. The system is comprised of instruments, single-use cartridges and a wireless communications link that conveys protocol information to the instruments from a Theranos Server and relays assay data to the Server for interpretation and distribution. Blood, plasma serum and control materials may be analyzed by the System. Calibration is performed at Theranos on a cartridge-lot-specific basis.

The System accepts a metered sample (25uL), from a proprietary sampling device or a pipette, dilutes it automatically to levels appropriate to each assay then executes an automated ELISA assay protocol. The protocol is selected from a set of released protocols available on the Theranos Server and identified by reading a bar code on each cartridge. The bar code is also linked to an assay lot-specific calibration algorithm. Assays are complete in about one hour.

Assays are typically grouped (multiplexed) in particular cartridges designed to monitor specific disease and therapeutic processes. For example, a cartridge designed to monitor acute and inflammatory processes measures IL-6, TNF-α and CRP. Schering-Plough is interested in use of the Theranos System and has sponsored a validation exercise at Theranos focused on the inflammatory marker cartridge.

In this exercise, many instruments (60) and three lots of cartridges were used.

2. Storage and Use

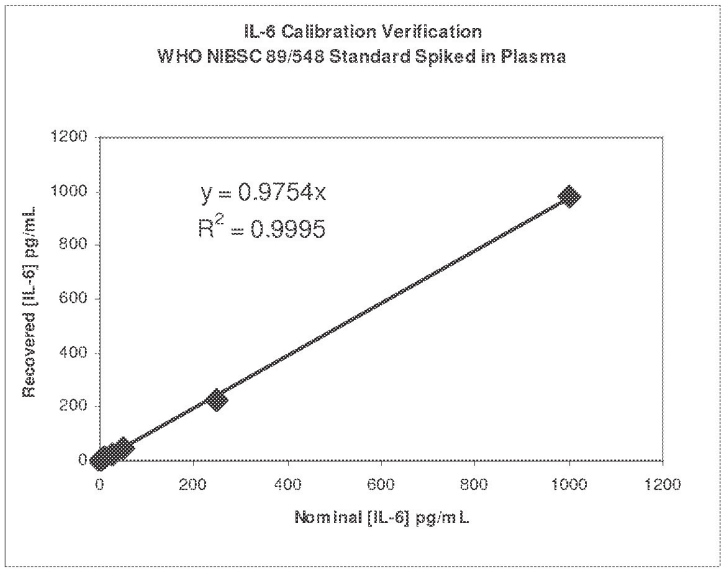
Theranos cartridges should be stored in the original unopened packaging in an upright position at 4°C. Theranos instruments require no user maintenance or calibration. User prompts are provided on a screen which is part of the instrument.

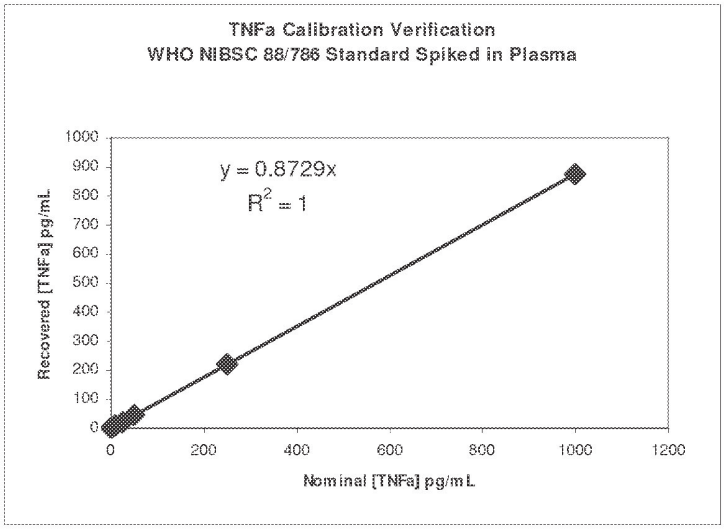
3. Calibration

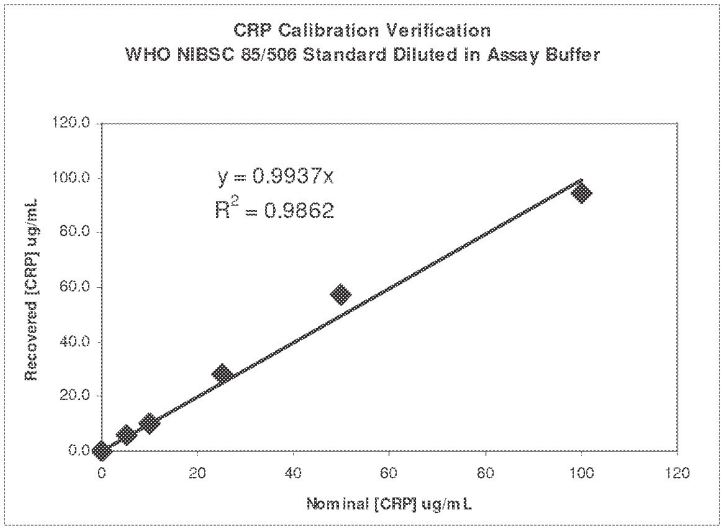
IL-6 and TNF-α assay calibration utilize recombinant analytes expressed in human-cell lines as calibration materials. These are reportedly more stable than recombinant analytes made in bacteria and more similar to the naturally occurring analytes. The CRP assay is calibrated with a human plasma-derived analyte. Theranos Systems assays recognize "natural", recombinant, and human-cell line expressed recombinant forms of IL-6 and TNF-α. Each lot of Theranos Cartridges is individually calibrated, the calibration equation is linked to the cartridge barcode and results are automatically computed on the Theranos data server. For this validation study, three cartridge lots were produced and calibrated.

NIBSC WHO Verification of Calibration

Exemplary assay responses are shown in Appendix A. Calibrations for IL-6, TNF-α and CRP were verified by testing the recovery of the current National Institute for Biological Standards and Control (NIBSC) World Health Organization (WHO) Reference Standards. The current WHO standard for IL-6 is NIBSC code 89/548 (recombinant protein produced in CHO cells with post translational modifications), for TNF-α NIBSC code 88/786 (a natural human protein derived from human BALL-1 cells), and for CRP NIBSC code 85/506 from human plasma. Spike recovery of all three WHO standards were within acceptable limits across the assay ranges as shown in the figures and tables below. Note that for the TNF-α assay we found low recovery (about 30%) of the WHO standard in a reference kit (R&D Systems Quantikine HS catalogue # HSTA00D, data shown in Appendix B). Therefore comparisons of sensitivity and slopes of assay correlations of results of the Theranos System with those of R&D Systems kits will show different results due to their respective calibrations. For example, the R&D Systems Assay would report a TNF-α value of 4 pg/mL when the Theranos Assay reports 12 pg/mL. If desired by a customer the Theranos System can be configured (in calibration algorithms) to provide results matching those of R&D Systems assays (or those of other predicate assay). It is our intention however to continue to perform primary calibration of Theranos assays using International Standard materials whenever possible since predicate assays not so calibrated may be subject to lot-to-lot variation in calibration.







Theranos Systems Recovery of IL-6 (NIBSC code 89/548) Spiked in Plasma
n=3 cartridges, 3 instruments per level

[IL-6] IU/mL	[IL-6] pg/ml	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
100	1000	981.1	11	980.1	98
25	250	227.1	16	226.2	90
5	50	45.2	10	44.2	88
3	25	21.5	8	20.5	82
1	10	10.5	9	9.5	95
0	0	1.0	47	0.0	N/A

Theranos Systems Recovery of TNF-α (NIBSC code 88/786) Spiked in Plasma
n=3 cartridges, 3 instruments per level

[TNFa] IU/mL	[TNFa] pg/mL	Recovered [TNF-α] pg/mL	CV %	Minus Endogenous	% Recovery
46.5	1000	873.4	3	873.0	89
11.6	250	218.7	3	218.3	96
2.3	50	44.0	10	43.5	96
1.2	25	20.9	22	20.4	95
0.5	10	10.9	19	10.5	100
0	0	0.4	14	0.0	N/A

Theranos Systems Recovery of CRP (NIBSC code 85/506) in Assay Buffer
n=3 cartridges, 3 instruments per level

[CRP] IU/mL	[CRP] ug/ml	Recovered [CRP] ug/mL	CV %	% Recovery
98	100	94.6	2	95
49	50	57.4	18	115
24.5	25	28.1	15	113
10	10	10.2	14	102
4.9	5	5.7	20	114
0	0	0.0	30	N/A

4. Range

Reportable ranges based on calibration to WHO standards determined for these assays are:

Assay	Low	High
IL-6	2 pg/mL	1000 pg/mL
TNF-α	4[1] pg/mL	1000 pg/mL
CRP	0.05 ug/mL	100 ug/mL

As shown below, all three tested lots support these ranges[2].

[1] Equivalent to 1 pg/mL in the R&D Systems assay calibrated using R&D Systems calibrators

[2] The lower limit of the reportable range of the TNF-α assay has been extended below the LLOQ so as not to restrict the reportable range too much. The LLOQ is higher than anticipated due to unexpectedly high imprecision of the assay in the cartridge lots used for validation compared with other cartridge lots used in pre-clinical work. We are presently investigating the root cause of this imprecision.

5. Quantitation Limits

Assay calibrations and determination of Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) were performed and analyzed by proprietary software. Assay responses were fitted by a four-parameter equation and LLOQ and ULOQ determined according to FDA criteria. Calibrators were run in triplicate on three days (consecutive or non-consecutive) on 36 instruments for a total of nine cartridges per level, at 12 levels.

Summary of Calibration Analysis for three Cartridge Lots

Lot 2455142005	**IL-6**	**TNF-α**	**CRP**
LLOQ	1.3 pg/mL	6.3 pg/mL	0.05 ug/mL
ULOQ	1000 pg/mL	1000 pg/mL	150 ug/mL
Lot 2455146006	**IL-6**	**TNF-α**	**CRP**
LLOQ	1.3 pg/mL	6.3 pg/mL	0.05 ug/mL
ULOQ	1000 pg/mL	1000 pg/mL	150 ug/mL
Lot 2455156002	**IL-6**	**TNF-α**	**CRP**
LLOQ	2.3 pg/mL	6.3 pg/mL	0.05 ug/mL
ULOQ	1000 pg/mL	1000 pg/mL	150 ug/mL

Limits of detection (LOD)

The range in the Limits of detection calculated as 2*Signal SD/Slope of dose response (Δsignal/Δconc) are reported for the three lots of Theranos cartridges. Comparison data are also given for R&D Systems assays Minimum Detectable Dose "MDD" (which is equivalent to LOD). In addition to the calibration issue for the R&D Systems TNF-α assay discussed above which gives a four-fold lower limit for R&D Systems, we believe the calculation of MDD performed by R&D Systems may be compromised (falsely low) by the inability of any known spectrometer to report optical density to the required precision needed to support the calculated values.

The CRP MDD reported by R&D Systems is highly misleading since it represents the concentration in the assay rather than in the sample (which "must be diluted" according to their package insert prior to assay). Note that the Theranos assay uses a sample which is diluted 5000-fold. If we compare the actual sensitivity *in the assay medium* the Theranos value would be about 0.006 ng/mL.

Assay System	IL-6 (pg/mL)	TNF-α (pg/mL)	CRP (ng/mL)
Theranos	0.9 – 1.5	3.7 – 5.2	28 - 31
R&D Systems	0.02 – 0.11	0.04 – 0.19	0.005 – 0.22
R&D Systems[3]		0.16 – 0.76	

[3] Recalculated to reflect calibration to WHO standard material

6. Precision and Accuracy

Plasma with low endogenous analyte levels was spiked with thee levels of the analytes were measured in 16 cartridges per level on 48 instruments. Recovery of the spiked analyte was good. Imprecision (% CV) ranged from 10 - 25 %. Note that the imprecision cited includes both instrument-instrument and cartridge-cartridge variance.

Spiked Plasma Samples (n=16 cartridges, n=48 instruments)

Nominal [IL-6] pg/mL	Recovered [IL-6] pg/mL	StDev	CV %	% Recovery
800.3	806.9	79.8	9.9	101
50.3	50.5	4.7	9.2	100
5.3	5.1	0.8	15.5	96
Nominal [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**StDev**	**CV %**	**% Recovery**
500.3	418.9	39.6	9.5	84
50.3	42.7	5.1	12.0	85
12.3	12.9	3.2	24.6	105
Nominal [CRP] ug/mL	**Recovered [CRP] ug/mL**	**StDev**	**CV %**	**% Recovery**
50.1	50.4	10.0	19.9	101
1.6	1.6	0.3	16.8	97
0.1	0.1	0.0	20.6	103

7. Specificity

Assays were tested for cross reactivity and interference by the factors listed below, at high, mid and low analyte levels. Potential cross-reactants were selected based on package inserts of recognized predicate methods and added at levels deemed to be higher than those likely to be found in clinical samples. No significant cross reactivity or interference was observed for any of the assays by any of the tested factors at all analyte levels tested.

IL-6 Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)					
Substance	[Test Substance] ng/mL	Target [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	% Recovery
Control	0	1000.3	1100.3	7.8	110
	0	90.3	95.8	16.6	106
	0	8.3	9.4	4.8	113
IL-1α	10	1000.3	939.2	2.9	94
	10	90.3	97.0	15.7	107
	10	8.3	9.0	6.9	108
IL-2	10	1000.3	1047.7	1.7	105
	10	90.3	86.7	9.4	96
	10	8.3	8.7	22.3	105
IL-3	10	1000.3	950.0	12.7	95
	10	90.3	91.9	4.6	102
	10	8.3	7.9	4.4	95
IL-4	10	1000.3	908.0	10.9	91
	10	90.3	79.9	16.7	88
	10	8.3	8.1	18.1	97
IL-6 sR	50	1000.3	914.9	18.0	91
	50	90.3	81.2	1.3	90
	50	8.3	8.0	29.0	96
IL-7	10	1000.3	895.0	10.0	89
	10	90.3	78.1	9.1	87
	10	8.3	8.2	9.4	99
IL-8	10	1000.3	927.8	9.7	93
	10	90.3	82.3	17.1	91
	10	8.3	8.4	17.6	101
IL-11	10	1000.3	897.5	12.5	90
	10	90.3	90.3	6.1	100
	10	8.3	7.9	2.2	95
IL-12	10	1000.3	837.6	8.4	84
	10	90.3	85.8	14.7	95
	10	8.3	6.8	18.1	82
CNTF	10	1000.3	900.6	8.4	90
	10	90.3	95.3	5.8	106
	10	8.3	8.9	22.4	107
G-CSF	10	1000.3	925.0	18.7	92
	10	90.3	90.2	12.8	100
	10	8.3	9.7	6.9	117

IL-6 Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)					
Substance	[Test Substance] ng/mL	Target [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	% Recovery
sgp130	1000	1000.3	895.5	17.0	90
	1000	90.3	88.6	2.0	98
	1000	8.3	9.4	3.2	114
LIF R	50	1000.3	895.2	2.8	89
	50	90.3	78.5	16.5	87
	50	8.3	8.9	19.8	107
OSM	10	1000.3	945.4	9.5	95
	10	90.3	77.1	10.0	85
	10	8.3	6.9	16.8	83
TNF-β	10	1000.3	919.6	8.6	92
	10	90.3	83.3	15.8	92
	10	8.3	9.4	7.8	113
IL-1β	10	1000.3	901.2	8.1	90
	10	90.3	85.7	17.6	95
	10	8.3	7.5	10.5	90
sTNF RI	10	1000.3	1025.2	9.2	102
	10	90.3	83.4	11.4	92
	10	8.3	9.4	16.5	114
sTNF RII	10	1000.3	963.3	13.8	96
	10	90.3	90.7	10.2	100
	10	8.3	9.3	21.0	112

TNF-α Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)					
Substance	[Test Substance] ng/mL	Target [TNFa] pg/mL	Recovered [TNFa] pg/mL	CV %	% Recovery
Control	0	900.3	883.7	4.1	98
	0	90.3	85.4	4.1	95
	0	8.3	8.3	40.4	100
IL-1α	10	900.3	849.1	5.5	94
	10	90.3	89.6	12.7	99
	10	8.3	8.8	16.0	106
IL-2	10	900.3	855.2	23.5	95
	10	90.3	90.8	7.9	101
	10	8.3	9.6	18.5	116
IL-3	10	900.3	836.5	23.5	93
	10	90.3	74.3	5.4	82
	10	8.3	8.2	29.2	98
IL-4	10	900.3	884.6	6.9	98
	10	90.3	89.5	8.5	99
	10	8.3	7.0	49.3	84
IL-6 sR	50	900.3	874.0	23.5	97
	50	90.3	77.8	13.8	86
	50	8.3	8.6	34.8	103
IL-7	10	900.3	871.9	6.3	97
	10	90.3	82.8	37.1	92
	10	8.3	7.6	22.9	91

TNF-α Assay Specificity Test in Spiked Plasma (n=3 cartridges, 3 instruments per level)					
Substance	[Test Substance] ng/mL	Target [TNFa] pg/mL	Recovered [TNFa] pg/mL	CV %	% Recovery
IL-8	10	900.3	774.4	1.8	86
	10	90.3	83.4	13.5	92
	10	8.3	7.9	12.6	95
IL-11	10	900.3	901.8	1.5	100
	10	90.3	90.7	19.6	100
	10	8.3	9.3	36.8	112
IL-12	10	900.3	770.9	7.3	86
	10	90.3	77.4	15.8	86
	10	8.3	7.9	56.7	96
CNTF	10	900.3	920.1	6.0	102
	10	90.3	82.5	9.7	91
	10	8.3	8.7	18.9	105
G-CSF	10	900.3	1052.6	3.7	117
	10	90.3	95.6	20.7	106
	10	8.3	9.1	9.6	110
sgp130	1000	900.3	891.3	16.8	99
	1000	90.3	93.8	9.1	104
	1000	8.3	10.1	25.1	122
LIF R	50	900.3	781.5	20.7	87
	50	90.3	87.3	15.2	97
	50	8.3	9.1	12.1	110
OSM	10	900.3	862.1	10.6	96
	10	90.3	85.2	23.8	94
	10	8.3	7.4	54.1	89
TNF-β	10	900.3	804.0	24.7	89
	10	90.3	90.7	16.4	100
	10	8.3	7.7	32.3	92
IL-1β	10	900.3	900.0	17.3	100
	10	90.3	83.1	16.6	92
	10	8.3	8.3	33.1	101
sTNF RI	10	900.3	833.0	21.8	93
	10	90.3	86.4	19.5	96
	10	8.3	6.7	21.6	80
sTNF RII	10	900.3	801.3	8.9	89
	10	90.3	93.6	3.0	104
	10	8.3	8.2	14.2	99

CRP Assay Specificity Test in Assay Buffer (n=3 cartridges, 3 instruments per level)					
Substance	**[Test Substance] ng/mL**	**Target [CRP] ug/ml**	**Recovered [CRP] ug/ml**	**CV %**	**% Recovery**
Control	0	50	53.0	16	106
	0	10	8.1	34	81
	0	0.75	0.7	13	91
Pentraxin-2/SAP	30	50	49.2	19	98
	30	10	8.9	9	89
	30	0.75	0.8	4	102
Pentraxin-3/TSG-14	10	50	40.6	7	81
	10	10	8.2	14	82
	10	0.75	0.7	5	100

8. Linearity

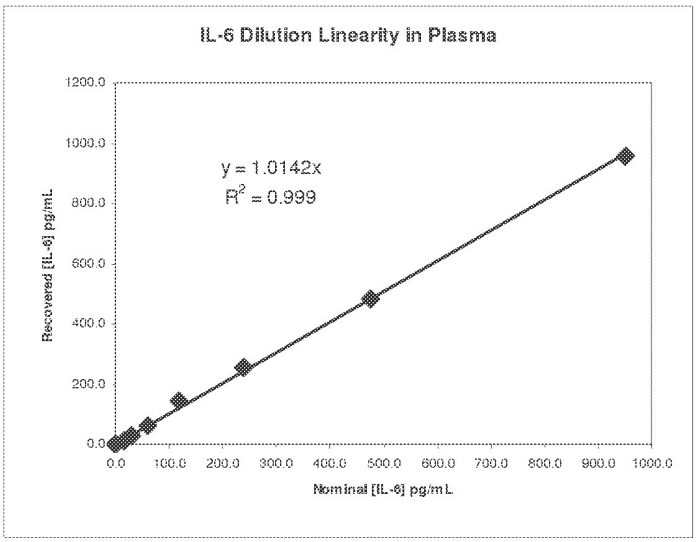
A plasma sample with low endogenous analyte levels was spiked with known levels of IL-6, TNF-α, and CRP then diluted serially with the unspiked plasma. All assays showed an appropriate linear dilution response across the dilution range (500 – 2000-fold). Data are tabulated and graphed below.

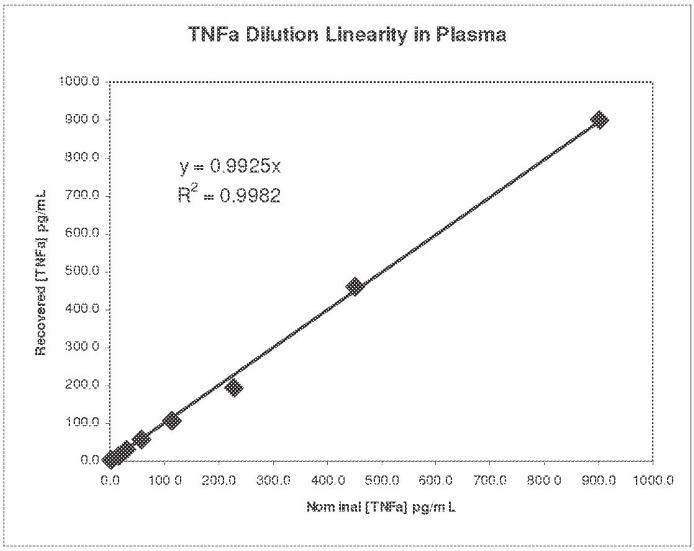
Dilution Linearity in Plasma, Multiplexed Assays (n=3 cartridges, 3 instruments per level)

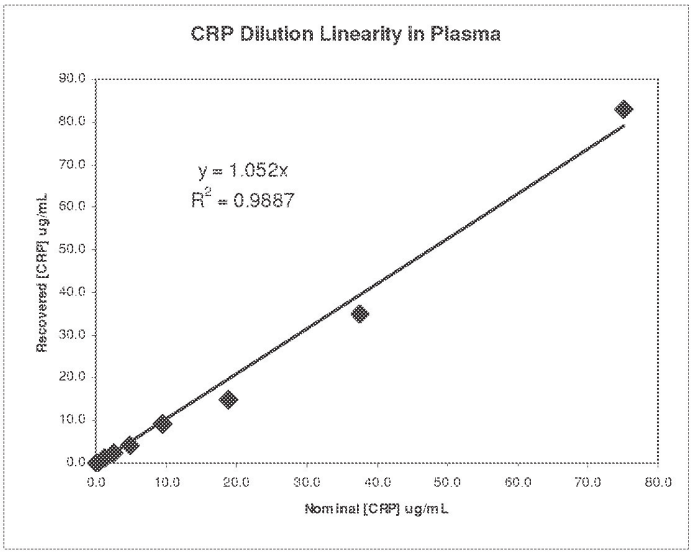
IL-6				
Spiked [IL-6] pg/mL	**[Expected] pg/ml**	**[Recovered] pg/mL**	**CV %**	**% Recovery**
950	950.5	958.1	7	101
	475.5	480.9	11	101
	238.0	256.1	18	108
	119.2	143.9	25	121
	59.8	62.3	3	104
	30.1	28.3	23	94
	15.3	13.3	34	87
	0.5	0.5	88	100

TNF-α				
Spiked [TNFa] pg/mL	**[Expected] pg/ml**	**[Recovered] pg/mL**	**CV %**	**% Recovery**
900	902.7	899.2	11	100
	452.7	461.5	9	102
	227.7	194.6	6	85
	115.2	105.0	11	91
	59.0	56.1	2	95
	30.9	30.6	4	99
	16.8	14.9	26	89
	2.7	2.7	14	100

CRP				
Spiked [CRP] ug/mL	**[Expected] ug/ml**	**[Recovered] ug/mL**	**CV %**	**% Recovery**
75	75.1	82.8	34	110
	37.6	35.0	0	93
	18.8	14.7	10	78
	9.5	9.1	12	96
	4.8	4.1	8	85
	2.4	2.4	7	98
	1.3	1.3	15	102
	0.1	0.1	29	100







9. Matrix Effects

Plasma or serum containing various potentially interfering factors or substances were spiked with known levels of analyte and the resulting recovery of the spiked analyte calculated after correction for endogenous analyte. None of the assays showed interference from icteric, hemolyzed, lipemic, or rheumatoid factor-positive samples as shown in the tables below

NORMAL SERUM Sample: PromedDx 10739123 (n=3 cartridges, 3 instruments per level)

Spiked [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
1000	1019.1	14	1015.82	102
250	224.9	4	221.58	89
50	47.7	14	44.42	89
25	25.3	6	22.01	88
10	12.6	9	9.29	93
0	3.3	43	0.00	
Spiked [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
1000	1019.1	14	1014.7	101
250	224.9	4	220.5	88
50	47.7	14	43.3	87
25	25.3	6	20.9	84
10	12.6	9	8.2	82
0	4.4	60	0.0	
Spiked [CRP] ug/mL	**Recovered [CRP] ug/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
100	107.4	11	107.3	107
50	49.3	13	49.3	99
25	25.0	23	24.9	100
10	9.6	41	9.5	95
5	5.9	17	5.8	116
0	0.1	12	0.0	

LIPEMIC SERUM Sample: Vital Products SFB8315 (n=3 cartridges, 3 instruments per level)

Spiked [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
1000	872.5	15	868.8	87
250	214.1	4	210.4	84
50	47.8	15	44.1	88
25	24.5	6	20.8	83
10	14.4	19	10.7	107
0	3.7	12	0.0	
Spiked [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
1000	965.0	17	962.8	96
250	230.8	15	228.6	91
50	56.6	40	54.4	109
25	25.4	13	23.2	93
10	14.8	14	12.6	126
0	2.2	32	0.0	
Spiked [CRP] ug/mL	**Recovered [CRP] ug/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
100	119.4	36	119.1	119
50	54.2	40	53.9	108
25	24.4	25	24.1	96
10	10.4	9	10.1	101
5	5.8	15	5.6	111
0	0.2	12	0.0	

HEMOLYZED PLASMA Sample: Stanford W070509118560 (n=3 cartridges, 3 instruments per level)

Spiked [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
1000	1010.9	10	1010.0	101
250	274.6	13	273.7	109
50	51.6	2	50.7	101
25	26.8	11	25.9	104
10	10.5	12	9.6	96
0	0.9	41	0.0	
Spiked [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
1000	898.7	14	895.1	90
250	223.5	12	219.9	88
50	44.2	11	40.6	81
25	27.7	23	24.1	96
10	12.0	23	8.4	84
0	3.6	14	0.0	
Spiked [CRP] ug/mL	**Recovered [CRP] ug/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
100	119.6	10	119.5	119
50	54.0	10	53.9	108
25	22.5	14	22.4	90
10	11.6	3	11.5	115
5	5.6	11	5.5	110
0	0.1	4	0.0	

ICTERIC SERUM Sample: PromedDx 10739123 (n=3 cartridges, 3 instruments per level)

Spiked [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
1000	986.0	9	983.4	98
250	282.4	12	279.7	112
50	55.8	10	53.2	106
25	28.1	7	25.4	102
10	11.8	16	9.2	92
0	2.6	53	0.0	
Spiked [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
1000	969.8	5	967.4	97
250	219.6	22	217.2	87
50	45.0	11	42.6	85
25	24.5	5	22.1	88
10	10.6	22	8.2	82
0	2.4	17	0.0	
Spiked [CRP] ug/mL	**Recovered [CRP] ug/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
100	109.5	8	108.4	108
50	41.7	80	40.6	81
25	29.6	14	28.4	114
10	10.1	11	9.0	90
5	6.4	19	5.3	106
0	1.1	3	0.0	

RHEUMATOID FACTOR POSITIVE SERUM Sample: Vital Products SFB7884 (n=3 cartridges, 3 instruments per level)

Spiked [IL-6] pg/mL	Recovered [IL-6] pg/mL	CV %	Minus Endogenous	% Recovery
1000	1118.0	10	1097.9	110
250	286.9	9	266.7	107
50	77.7	13	57.6	115
25	46.3	12	26.2	105
10	30.4	6	10.2	102
0	20.1	6	0.0	
Spiked [TNFa] pg/mL	**Recovered [TNFa] pg/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
1000	1116.4	11	1112.3	111
250	228.9	5	224.8	90
50	48.0	13	43.9	88
25	24.2	13	20.1	80
10	14.0	20	9.9	99
0	4.1	27	0.0	
Spiked [CRP] ug/mL	**Recovered [CRP] ug/mL**	**CV %**	**Minus Endogenous**	**% Recovery**
100	110.9	18	105.8	106
50	49.1	17	44.0	88
25	34.2	29	29.0	116
10	15.5	9	10.3	103
5	10.9	11	5.7	114
0	5.2	28	0.0	

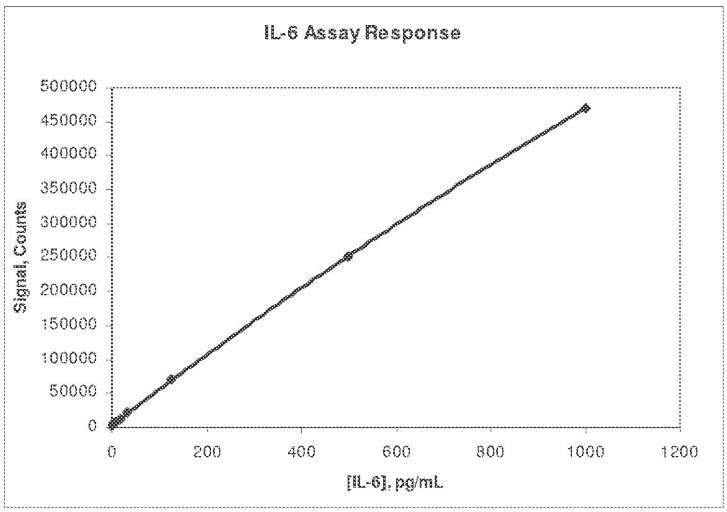
10. Stability

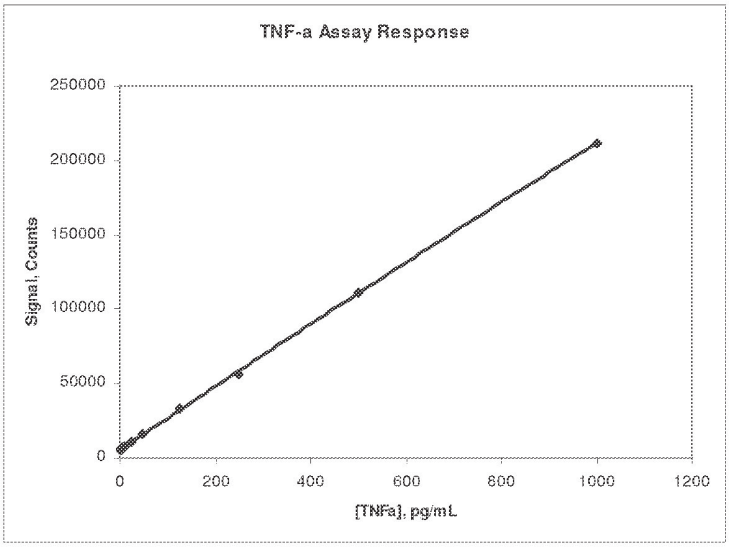
The stability of component reagents for the present assays has been studied individually in lots made previous to the present study. The capture surfaces were stable for over 12 months, and the detection conjugates for at least six months. Stability of the integrated cartridges used for this validation report stored at 4C is being monitored and an updated report will include this data. Cartridges are initially assigned an expiry date of three months post manufacture.

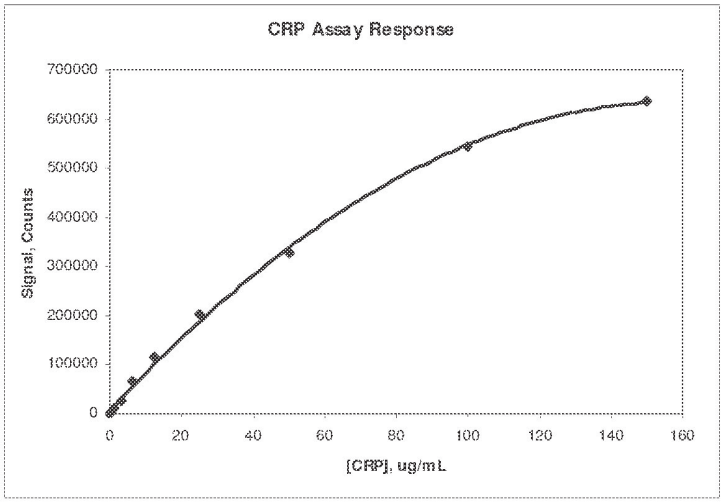
Conclusions:

The Theranos IL-6, TNF-α, CRP assay multiplex has been shown to give accurate and precise results for three independently calibrated cartridge lots and all the many instruments used. Assay calibration has been established using WHO or other standard materials. Lower and upper levels of quantitation have been established. The assays are specific for their respective analytes when tested against potential cross reactants and are not interfered with by agents that may cause problems in immunoassays. Dilution linearity is satisfactory for all the assays. Assay cartridge stability studies are underway.

Appendix A







Appendix B

Comparison of Theranos Systems TNFa Calibration to Other Available Commercial Methods

Plasma samples were spiked with WHO TNF-a Standard (NIBSC code 88/786) and run in Theranos Systems and in R&D Quantikine High Sensitivity Human TNF-α ELISA (catalogue # HSTA00D). The results are shown below.

THERANOS SYSTEMS Recovery of TNFa WHO Standard Spiked in Plasma

Nominal Spike		1pg/mL = 0.0465 IU/mL			
[TNFa] IU/ml	[TNFa] pg/ml	Calc. pg/mL	Minus Endogenous	Calc. IU/mL	% Recovery
0	0	5.2	0.0		
0.1	2.5	8.1	2.9	0.1	118
0.2	5	11.5	6.3	0.3	126
0.5	10	14.9	9.7	0.5	97
1.2	25	35.9	30.8	1.4	123
2.3	50	57.6	52.4	2.4	105
11.6	250	217.6	212.5	9.9	85
46.5	1000	1120.6	1115.4	51.9	112

R&D QUANTIKINE HS ELISA Recovery of TNFa WHO Standard Spiked in Plasma					
Nominal Spike		1pg/mL = 0.0465 IU/mL			
[TNFa] IU/ml	[TNFa] pg/ml	Calc. pg/mL	Minus Endogenous	Calc. IU/mL	% Recovery
0	0	0.2	0.0		
0.1	2.5	1.0	0.8	0.04	32
0.2	5	1.8	1.6	0.07	32
0.5	10	3.2	3.0	0.14	30
1.2	25	7.3	7.1	0.3	28
2.3	50	15.0	14.8	0.7	30
11.6	250	83.6	83.4	3.9	33
46.5	1000	308.0	307.7	14.3	31