To: 'Cullen, Constance'[constance.cullen@spcorp.com]
Cc: Gary Frenzel[gfrenzel@theranos.com]; Elizabeth Holmes[eholmes@theranos.com]
From: Carolyn Balkenhol[/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=CBALKENHOL]
Sent: Sat 12/13/2008 1:12:35 AM (UTC)
Subject: RE: Follow up to our call
Assay Validation - Human IL-6.pdf
Assay Validation - Human TNF-a.pdf
Assay Validation - GIP.pdf
Assay Validation - GLP-1.pdf

Connie,

Well, it was slightly longer than the one day I promised – but here you go. I've attached the Validation Summary Reports for IL-6 and TNF-a, which you requested – and I've also included a couple others, so you get a feel for the breadth of our technical capabilities. Please let me know if there's anything else we can provide.

We're looking forward to your January visit to Theranos. Let me know if I can be of help with that.

Have a great weekend.
Carolyn

From: Cullen, Constance [mailto:constance.cullen@spcorp.com]
Sent: Wednesday, December 10, 2008 1:23 PM
To: Elizabeth Holmes
Cc: Carolyn Balkenhol; Gary Frenzel
Subject: RE: Follow up to our call

Elizabeth,
I was wondering if you could provide your validation reports on any of these assays? The reports that you provided showed correlations between your assays and corresponding commercial assays which is important, but I was more interested in the validation reports for your assays.

Interleukin-6 and TNF are of particular interest since these would certainly be candidates in a rheumatoid arthritis model.

Thanks. Connie

-----Original Message-----
From: Elizabeth Holmes [mailto:eholmes@theranos.com]
Sent: Wednesday, November 12, 2008 4:53 PM
To: Cullen, Constance
Cc: Carolyn Balkenhol; Gary Frenzel
Subject: Follow up to our call

Connie,

Great to connect. We have attached several reports from programs we have done with other pharmaceutical companies.

We are looking forward to hearing your thoughts on which assays will be most relevant for the inflammatory agents' upcoming trials.

Elizabeth.

Elizabeth Holmes
President and CEO
Theranos, Inc.

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Theranos Assay System Validation Summary Report
Human IL-6 Assay

Assay Specification: The assay is designed to measure active IL-6 in human blood, plasma and serum. Reportable ranges and limits of detection are:

Sample type	Low (pg/mL)	High (pg/mL)
Human plasma	0.1	1156
Human serum	0.1	1156
Human blood	0.1	1156

Specificity: This assay recognizes both natural and recombinant human IL-6 and does not significantly cross react with human IL-6 sR, IL-6 sR/sgp130, IL-9, IL-10, IL-11, IL-12, IL-12/23 p40, LIF, LIF R, or CD40 Ligand.

Precision: 8 samples were assayed in replicate (N = 3) on multiple instruments (N = 2).

Inter-run (on one instrument) CV:	5.7 %
Inter-instrument CV:	3.8 %
Total CV (any cartridge, any instrument):	7.0 %
Day-day CV = Inter-run CV	

Sensitivity: Limit of detection in plasma (95% confidence) = 0.13 pg/mL

Dilution linearity: Five plasma samples were spiked with IL-6 then diluted to the specified target concentrations with the results shown below. The IL-6 values from the Theranos system agree within <10% of the target.

Plasma Sample	Target (pg/mL)	Result (pg/mL)
1	1157.3	1053.6
	232.7	242.5
	59.3	57.9
	13.1	11.8
	5.4	4.8
	3.4	3.3
	2.5	2.2
2	1155.8	1171.3
	231.2	253.7
	57.9	58.4
	11.7	10.5
	4.0	4.3
	2.0	2.1
	1.1	1.0
3	2.4	2.6
	1.4	1.4
4	3.0	2.9
	2.0	2.0
5	3.1	3.1
	2.1	2.0

Interfering substances: The assay was tested for Rheumatoid Factor interference. No interference from Rheumatoid Factor occurred.

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Theranos Assay System Validation Summary Report
Human TNF-α Assay

Assay Specification: The assay is designed to measure active TNF-α in human blood, plasma and serum. Reportable ranges and limits of detection are:

Sample type	Low (pg/mL)	High (pg/mL)
Human plasma	1.6	17,662
Human serum	2.0	17,662

Specificity: This assay recognizes both natural and recombinant human TNF-α and does not significantly cross react with human: TNF-β, TNF RI, TNF RII, and TNF RII/Fc Chimera. It does not cross react with mouse, rat or canine TNF-α.

Precision: 8 samples were assayed on multiple instruments (N = 3).

Inter-instrument CV:	6.4 %
Total CV (any cartridge, any instrument):	6.4 %

Sensitivity: Limit of detection in plasma (95% confidence) = 1.3 pg/mL.

Dilution linearity: A pooled plasma sample was spiked with TNF-α then diluted to the specified target concentrations with the results shown below. The TNF-α value from the Theranos system agree within <10% of the target.

Target (pg/mL)	Result (pg/mL)
422.1	433.1
85.7	86.6
22.6	21.6
8.6	8.6
4.0	4.3
1.6	1.6

Interfering substances: The assay was tested for Rheumatoid Factor interference. No interference from Rheumatoid Factor occurred.

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Theranos Assay System Validation Summary Report
GIP Assay

Assay Specification: The assay is designed to measure active GIP in human and selected animal blood, plasma and serum. Reportable ranges and limits of detection are:

Sample type	LOD (pg/mL) – 95% conf.	Low (pg/mL)	High (pg/mL)
Human plasma	1.4	2	2000
Human serum	1.4	2	2000
Human blood	2.0	3	3000

Calibration: Assay calibration is traceable to authentic human GIP.

Specificity: The antibody pair used in this assay is specific to human, rat and mouse GIP and does not significantly cross react with Glucagon, Oxynlomodulin, GLP1, and GLP2

Precision: Replicate measurements were made for 13 serum and plasma samples (Analyte range: 26 – 1663 pg/mL) in two Theranos instruments. Total CV across cartridges and instruments averaged 6%.

Accuracy: Thirteen serum and plasma samples from diabetic human subjects were analyzed in a commercially available reference assay and the Theranos system with the following results:

Analyte range: 26 – 1663 pg/mL
Theranos (y) = 0.8081 Ref (x) - 20.2; R^2 = 0.9962

Note that the system could be calibrated to match the predicate assay results so the slope of the regression would be 1.00.

Dilution linearity: GIP in a serum-like matrix was diluted to several target levels and measured. Recovery of analyte is reported below.

GIP target (pg/mL)	GIP result (pg/mL)	Analyte recovery (%)
15	13.1	87.1
120	130.3	108.6
940	985.3	104.8

Interfering substances: None evident from clinical studies <work in progress>.

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Theranos Assay System Validation Summary Report
GLP-1 Assay

Assay Specification: The assay is designed to measure active GLP-1 in human and selected animal blood and plasma. Reportable ranges are:

Sample type	Low (pM)	High (pM)
Human plasma	0.5	125[1]
Human blood	0.6	150
Dog plasma	6.0[2]	600

Specificity: The assay measures human and animal GLP-1(7-37) and GLP-1(7-36) amide and has <0.2% cross-reactivity with GLP-1(1-37), GLP-1(9-36) amide, glucagon, human GIP and exendin-4. Human GLP-2 cross-reacts approximately 1%. The assay has also been calibrated for active GLP-1 of other species (for example, dog).

Calibration: Assay calibration is traceable to authentic synthetic *active* GLP-1. The assay is calibrated to provide results equivalent to those of a reference commercially available immunoassay for the same analyte.

Precision: Plasma samples were assayed in replicate (N = 4) on multiple instruments (N = 6). Assay *concentration* CV is constant over the range 5 – 125 pM.

Inter-run (on one instrument) CV:	7.6 %
Inter-instrument CV:	4.4 %
Total CV (any cartridge, any instrument):	8.7 %
Day-day CV = Inter-run CV	

Sensitivity: Limit of detection in plasma (95% confidence) = 0.5 pM

Accuracy: The assay was used for eight dog plasma samples having GLP-1 levels ranging from 6 – 600 pM with the following results:

Theranos (y) = 1.0088*Reference method (x) - 0.78; R = 0.999.
R^2 = 0.9989. The difference between the system and reference results was less than 10% for all samples.

Dilution linearity: Two plasma samples were spiked with GLP-1 then diluted to the specified target concentrations with the results shown below. The GLP-1 values from the system agree within < 7 % of the target.

Target level (pM)	Result Plasma 1	Result Plasma 2
62.5	59.9	67.1
15.6	15.8	14.9

Interfering substances: None evident from clinical studies <work in progress>.

[1] The assay is linear to much higher levels (as shown by the results with dog plasma), but the initial calibration for human plasma was to 125 pM. It is expected that the assay will be able to measure up to at least 600 pM.
[2] No lower samples were available for calibration; the assay is expected to be able to measure down to 0.5 pM.