From: Kapil Gadkar <kgadkar@theranos.com>
Sent: Thursday, July 14, 2011 2:33 PM
To: Victoria Sung <VSung@celgene.com>
Cc: Daniel Young <dyoung@theranos.com>
Subject: RE: PD Marker Assays
Attach: Theranos IGF-1 Validation Report.pdf

Hi Vicki,

Attached is the report for IGF-1. Plz let me know if you have any questions.

Cheers
Kapil

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Thursday, July 07, 2011 1:57 PM
To: Daniel Young
Cc: Kapil Gadkar; Hem Singh; Lisa Serme
Subject: RE: PD Marker Assays

Hi Daniel and Kapil,
Just inquiring as to when we might have a status update on the assays in development for ACE-011 REN-001?
Thank you and regards,
Vicki

From: Daniel Young [mailto:dyoung@theranos.com]
Sent: Wednesday, June 15, 2011 2:00 PM
To: Victoria Sung
Cc: Kapil Gadkar
Subject: RE: PD Marker Assays

Hi Vicki,

Sorry for the delay in providing updates. We had been waiting for Elizabeth and Randall to connect, which they have done just recently. As Elizabeth mentioned to Randall, we are highly focused on supporting the next phase of the study in October, and we will be providing further assay development details in the next week.

The reports for IGF1 and BSAP will be communicated to you very shortly.

Thanks,
Daniel

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Tuesday, June 07, 2011 10:59 AM
To: Kapil Gadkar
Cc: Daniel Young
Subject: RE: PD Marker Assays

Hi Kapil,

Just wondering if you have reports for IGF1 and BSAP available now? Also, the ACE-011 team is still eagerly awaiting

some sort of update with regard to the status of the other PD marker assays. Elizabeth did not respond to e-mails from me or Randall last month…do you forsee that there may be an issue with completing the assays by October? If so, we really do need know in advance; the long silence from Theranos has begun to worry many of us; from an operational standpoint, we really do need to be prepared to move forward regardless of which assays are available.

I appreciate your help.
Regards,
Vicki

From: Kapil Gadkar [mailto:kgadkar@theranos.com]
Sent: Tuesday, May 17, 2011 4:01 PM
To: Victoria Sung
Cc: Daniel Young
Subject: RE: PD Marker Assays

Hi Vicki,

The assay development for IGF1 and BSAP were completed last year. The internal reviews of the validation reports were not completed at that time. I will have them done and communicate the reports of these to you first week in June.

Cheers
Kapil

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Tuesday, May 10, 2011 2:29 PM
To: Kapil Gadkar
Subject: RE: PD Marker Assays

Hi again,
I found the e-mail chain below from last November. At the time, you mentioned that the IGF1 and BSAP assays were completed; however we didn't receive reports for these. If you could send those to me while you are inquiring about the others, I would very much appreciate it.
Thanks,
Vicki

From: Kapil Gadkar [mailto:kgadkar@theranos.com]
Sent: Monday, November 15, 2010 5:23 PM
To: Victoria Sung
Subject: RE: PD Marker Assays

Thanks. Let me know if the Celgene team have any questions/thoughts.

Kapil

Kapil Gadkar
Principal Scientist
Computational Biosciences
Theranos Inc.
3200 Hillview Ave.
Palo Alto, CA 94304
+1.650.320.2715

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Monday, November 15, 2010 5:20 PM
To: Kapil Gadkar
Subject: RE: PD Marker Assays

Hi Kapil,
Thank you so much for the update. I will convey this to the project team at our meeting tomorrow.

Yes, EPO and Hgb are definitely two of the highest priority markers (in addition to FSH, which is already developed). Hepcidin is also very important to us but I think EPO and Hgb are at the top of the list. Glad that you can purchase testosterone now. Please let me know if you need anything from Celgene.

Best regards,
Vicki

From: Kapil Gadkar [mailto:kgadkar@theranos.com]
Sent: Monday, November 15, 2010 5:15 PM
To: Victoria Sung
Subject: RE: PD Marker Assays

HI Vicki,

We have finished development of the assays of IGF-1 and BSAP. We will communicate the validation reports to you shortly.
Assays for hemoglobin, EPO, FGF-23, PTH, TRAP5b, hepcidin and osteocalcin are at different stages of development. Most of these are planned to be deployed at the start of part 2; the remaining assays can be deployed soon after. Also, we now have clearance to directly obtain testosterone for development of this assay – thanks for the offer.

Based on prior discussions, our understanding is that hemoglobin, EPO and hepcidin are of highest priority to you. Is this still consistent with your current thinking?

Apologies for the delayed response. Let me know if you have any questions.

Cheers
Kapil

Kapil Gadkar
Principal Scientist
Computational Biosciences
Theranos Inc.
3200 Hillview Ave.
Palo Alto, CA 94304
+1.650.320.2715

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Thursday, November 11, 2010 3:05 PM
To: Kapil Gadkar
Subject: RE: PD Marker Assays

Hi Kapil,
Hope things are going well. I was wondering if you've had a chance to follow up with the assay team with regards to ongoing ACE-011 activities? I am curious about how the hepcidin assay, in particular, is going.

Thanks very much.
Regards,
Vicki

From: Kapil Gadkar [mailto:kgadkar@theranos.com]
Sent: Wednesday, October 27, 2010 10:57 AM
To: Victoria Sung
Subject: RE: PD Marker Assays

Hi Vicki,

I will follow up with the assay team and get back to you in a day or so. I will send you the reports that are ready and as they become ready.

Cheers
Kapil

Kapil Gadkar
Principal Scientist
Computational Biosciences
Theranos Inc.
3200 Hillview Ave.
Palo Alto, CA 94304
+1.650.320.2715

From: Victoria Sung [mailto:VSung@celgene.com]
Sent: Tuesday, October 26, 2010 5:15 PM
To: Kapil Gadkar
Subject: PD Marker Assays

Hi Kapil,
Hope you're doing well. I just thought I'd check in with you to see how development of the other PD markers is going? If there are reports ready, it would be helpful to receive them gradually over the course of the next few months rather than in a batch at the end…

Also, do you know if the research team has tackled the hepcidin assay yet? And do they have testosterone for development of the assay? Please let me know if the assay development group has any questions/requests from us.

Thanks!
Regards,
Vicki

****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED
INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL
OR INDIVIDUALS NAMED ABOVE.
If the reader is not the intended recipient, or the
employee or agent responsible to deliver it to the
intended recipient, you are hereby notified that any
dissemination, distribution or copying of this
communication is strictly prohibited. If you have
received this communication in error, please reply to the
sender to notify us of the error and delete the original
message. Thank You.
****
****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED

****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED
INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL
OR INDIVIDUALS NAMED ABOVE.
If the reader is not the intended recipient, or the
employee or agent responsible to deliver it to the
intended recipient, you are hereby notified that any
dissemination, distribution or copying of this
communication is strictly prohibited. If you have
received this communication in error, please reply to the
sender to notify us of the error and delete the original
message. Thank You.
****
****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED
INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL
OR INDIVIDUALS NAMED ABOVE.
If the reader is not the intended recipient, or the
employee or agent responsible to deliver it to the
intended recipient, you are hereby notified that any
dissemination, distribution or copying of this
communication is strictly prohibited. If you have
received this communication in error, please reply to the
sender to notify us of the error and delete the original
message. Thank You.
****

****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED
INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL
OR INDIVIDUALS NAMED ABOVE.
If the reader is not the intended recipient, or the
employee or agent responsible to deliver it to the
intended recipient, you are hereby notified that any
dissemination, distribution or copying of this

communication is strictly prohibited. If you have
received this communication in error, please reply to the
sender to notify us of the error and delete the original
message. Thank You.
****

****
THIS ELECTRONIC MAIL MESSAGE AND ANY ATTACHMENT IS
CONFIDENTIAL AND MAY CONTAIN LEGALLY PRIVILEGED
INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL
OR INDIVIDUALS NAMED ABOVE.
If the reader is not the intended recipient, or the
employee or agent responsible to deliver it to the
intended recipient, you are hereby notified that any
dissemination, distribution or copying of this
communication is strictly prohibited. If you have
received this communication in error, please reply to the
sender to notify us of the error and delete the original
message. Thank You.
****

IGF-1 Assay Validation Report

Theranos, Inc.

July, 2011

This Validation Report contains Theranos Confidential Information and is being provided to Celgene under the parties' Mutual Confidentiality Agreement. Celgene may provide this Report to B2S Consulting -- specifically and only to Dr. Bowsher – under the parties' three-way Unilateral Disclosure Agreement for the limited purposes set forth therein. Any further dissemination, use or disclosure of the Report, in whole or in part, is strictly prohibited.

theranos®
redefining healthcare

TABLE OF CONTENTS

Analyte Background

IGF-1 is a polypeptide protein hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults. It consists of 70 amino acids in a single chain with three intra-molecular disulfide bridges and has a molecular weight of 7,649 Daltons. IGF-1 is produced primarily by the liver as an endocrine hormone. Its production is stimulated by growth hormone and can be retarded by under-nutrition, growth hormone insensitivity, lack of growth hormone receptors, or failures of the downstream signaling pathways. Approximately 98% of IGF-1 is bound to one of 6 binding proteins (IGF-BP). IGFBP-3, the most abundant of these, accounts for 80% to 90% of all IGF binding. IGF-1 binds to IGFBP-3 in a 1:1 molar ratio.

Clinical Range

IGF-1 levels can be measured in the blood in 10-1000 ng/mL amounts. However levels of IGF-1 change significantly over the course of a person's life and are influenced by a number of factors. Clinically significant conditions and changes may be masked by the wide normal ranges. Factors that are known to cause variation in the levels of growth hormone (GH) and IGF-1 in the circulation include: genetic make-up, the time of day, age, sex, exercise status, stress levels, nutrition level and body mass index (BMI), disease state, race, estrogen status and xenobiotic intake.
Levels of IGF-1 range in Renal Failure patients range from 160 to 780 ng/mL with a mean of 425ng/mL.

Assay Specifications

IGF-1 is bound to several binding proteins in plasma. According to the literature, approximately 98% of IGF-1 is bound to one of 6 binding proteins (IGF-BP). IGFBP-3, the most abundant of these, accounts for 80% to 90% of all IGF binding. IGF-1 binds to IGFBP-3 in a 1:1 molar ratio. The capture antibody on the solid phase of this ELISA is specific to the IGF-1 antigen, while the solution phase detector antibody is specific to the IGF-1 binding protein 3.

The Theranos IGF-1 assay can measure IGF-1 in whole blood, plasma, and serum. In measuring analytes in blood two factors have an impact. (1) Most biomarkers are found in the plasma fraction of blood and so when a fixed volume of blood is sampled, the quantity of analyte is a function of the hematocrit. This effect is calibrated out since the hematocrit varies within a small range. (2) Some analytes bind to red cells (IGF-1 appears to be in this class).

For IGF-1 assay, calibration is performed in assay buffer; further, a plasma and whole blood correction is identified to be used based on the sample type. ULOQ and LLOQ in assay buffer are 1200ng/mL and 5ng/mL respectively.

Reference Assay and Controls

R&D Systems IGF-1 Elisa Kit (Cat# dg100) was utilized as the reference assay. WHO controls for IGF-1 (NIBSC code: 91/554) were utilized as controls.

Cross Reactivity

Cross reactivity was evaluated with associated analytes over appropriate ranges for each on the Theranos Assay System. All analytes tested produced no cross-reactivity (see Table below).

Table 1: Cross reactivity of Theranos IGF-1 assay

Test analyte	Analyte Level	% cross reactivity
Insulin (pg/mL)	5000	OORL
	2500	OORL
	250	OORL
	125	OORL
	75	OORL
	0	OORL
IGFBP2 (ng/mL)	600	OORL
	300	OORL
	150	OORL
	75	OORL
	37.5	OORL
	0	OORL
IGFBP3 (ng/mL)	600	OORL
	300	OORL
	150	OORL
	75	OORL
	37.5	OORL
	0	OORL
FGF (pg/mL)	800	OORL
	400	OORL
	200	OORL
	100	OORL
	40	OORL
	0	OORL
IGF-2 (ng/mL)	600	OORL
	300	OORL
	150	OORL
	75	OORL
	37.5	OORL
	0	OORL

Interference

Five substances (Insulin, IGFBP2, IGFBP4, FGF, IGF2) that are similar to IGF-1 were tested for interference in the assay. Percent recoveries of five calibrator levels of IGF-1 were calculated in presence of the interfering substances at concentrations at least 3x the highest endogenous levels. Interference is reported as a % relative to control.

Table 2: Recovery of IGF-1 in presence of interfering substances (percent relative to control)

		Insulin (15 ng/mL)	IGFBP2 (2 µg/mL)	IGFBP4 (2 µg/mL)	FGF (1.4 ng/mL)	IGF2 (2 µg/mL)
IGF-1 calibrators (ng/ml)	600	104	104	100	102	77
	300	106	101	108	102	79
	150	106	110	99	93	75
	75	112	110	101	97	72
	37.5	111	102	99	94	72

Precision

Precision across 3 reagent lots

The precision across three reagent lots is evaluated. Each IGF-1 calibrator was assayed in triplicates in each lot. An individual cartridge included the assay in replicates. 8 calibrator levels (inclusive of LLOQ & ULOQ) were included in the analyses. Calibrators above ULOQ and below LLOQ were included in determining the calibration curve.

A 4 PL calibration curve was determined using Theranos proprietary software. The assay range is determined to be 10-1200 ng/mL. Table 3 shows the performance of the IGF-1 assay in terms of %Recovery and %CV.

Table 3: Precision across 3 reagent lots

IGF-1 Calibrators (ng/mL)	Calc. conc. (lot 1)	Calc. conc. (lot 2)	Calc. conc. (lot 3)	combined % Recovery	combined % CV
1200	1140	1205	1086	95	5
600	618	652	634	106	3
300	309	303	315	103	2
150	144	145	146	97	1
75	72	66	66	91	5
38	39	37	43	104	8
19	20	21	24	114	10
10	10	12	12	111	10

The acceptance criteria are satisfied and precision across reagent lots for IFG-1 Theranos assay is established.

Precision across instruments

A midrange calibrator of IGF-1 (150 ng/mL) was assayed across 24 instruments to determine system %CV. Table 4 includes the average % recovery and % CV across the instruments tested.

Table 4: Precision across instruments

Instrument #	Calc. concentration	Instrument #	Calc. concentration	Instrument #	Calc. concentration
1	186.4	9	143.6	17	178.6
2	132.1	10	136.5	18	163.6
3	162.9	11	150.4	19	171.6
4	171.3	12	146.5	20	173.2
5	168.2	13	149.8	21	175.0
6	148.6	14	155.1	22	NA
7	134.8	15	151.1	23	170.6
8	155.4	16	155.7	24	152.8

The average % recovery across the instruments is 105% and the system % CV is 9.3%.

Plasma and Whole Blood Recovery

3 levels of calibrators of IGF-1 are spiked into plasma and whole blood samples to determine a correction for serum and whole blood. This correction is incorporated into the calibration equation when assaying the corresponding sample type.

Table 5: Plasma and whole blood recovery

		Plasma	Whole blood
IGF-1 calibrator (ng/mL)	Sample #	Calc. concentration*	Calc. concentration*
600	1	405.0	266.3
	2	436.5	312.5
	3	346.1	191.2
	4	318.4	238.6
300	1	219.6	149.3
	2	261.2	165.2
	3	176.7	91.8
	4	143.7	145.1
150	1	88.3	95.4
	2	77.0	119.9
	3	NA	32.1
	4	72.9	72.3

* calculated concentrations reported after correction with endogenous levels

An average correction is determined to be 1.68 for plasma samples and 2.38 for whole blood samples.

Selectivity in Whole Blood

10 whole blood samples (5 male and 5 female) are spiked with 3 calibrators of IGF-1 and assayed on the Theranos system. Table 6 shows the % recovery determined for the assays. The average recovery is close to 100% over 10 blood samples and three analyte levels. Variability in response is primarily due to reasons discussed in the assay specification section.

Table 6: Percent recovery for 10 whole blood samples spiked with IGF-1 calibrators

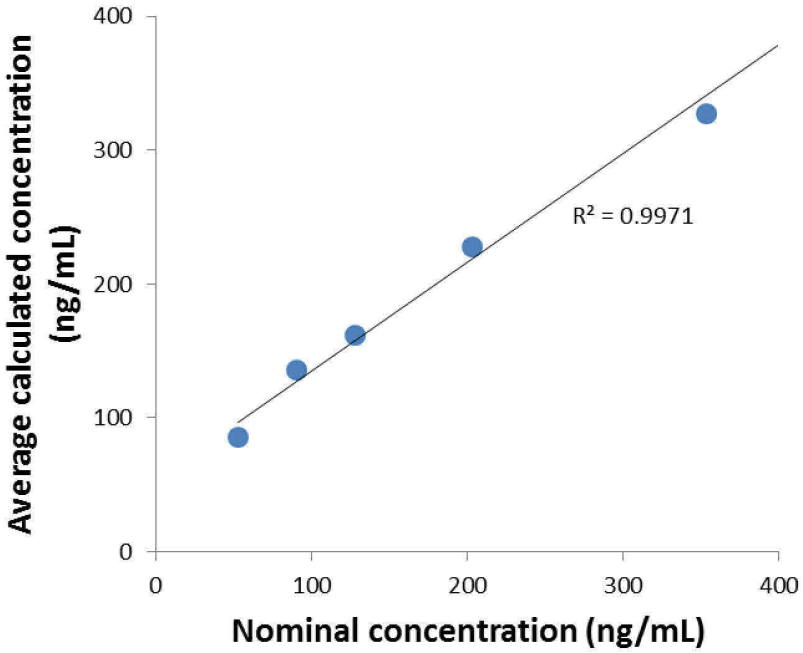
	% recovery in whole blood samples									
IGF-1 calibrators	1	2	3	4	5	6	7	8	9	10
1200	115	97	85	117	*65*	97	85	96	*151*	*158*
600	127	82	83	124	73	108	114	116	*163*	*139*
300	90	*56*	87	*42*	*143*	115	150	93	111	85

Dilution Linearity

A 600ng/mL calibrator is spiked clinical serum sample with low endogenous IGF-1 concentration (53 ng/mL). The spiked solution is diluted with the serum sample for obtaining serial dilution samples. Each sample is assayed in triplicates to determine average calculated concentration. Comparison between calculated concentrations and nominal concentrations is shown below.

Table 7: Nominal and calculated concentration from dilution linearity study

Nominal concentration	Calculated concentration
653	588
353	327.6
203	228
128	161.9
90.5	136.2
53	85.4



Extended Range

The assay response is determined at concentration of 2x of ULOQ (2400 ng/mL) to evaluate for potential hook effects. The response for samples with IGF-1 concentration of 2400 ng/mL is OORH. This established absence of any hook effect.

Validation in Clinical Samples

Clinical samples (n=28) are assayed on the Theranos systems and the IGF-1 R&D kit. Figure 3 shows the correlation between the two assay systems. In biological samples, more than 98% of IGF-1 is bound to one of six binding proteins. The most abundant binding protein is IGFBP-3. All commercial ELISA kits for IGF-1 include an acid solution separation step in their procedures to measure the free IGF-1 protein. The Theranos assay is designed to measure the bound IGF-1 and IGFBP-3 complex. The capture antibody on the solid phase of this ELISA is specific to the IGF-1 antigen, while the solution phase detector antibody is specific to the IGF-1 binding protein 3.

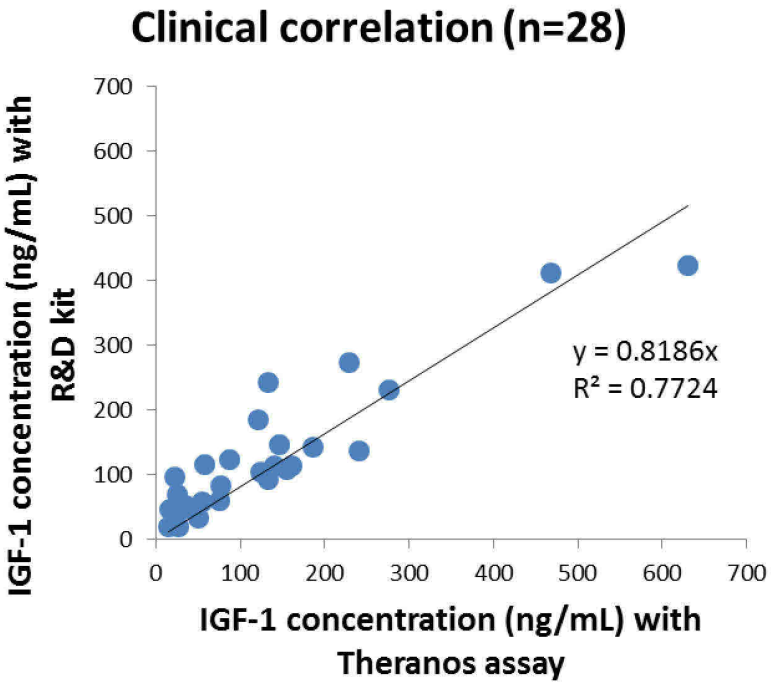


Figure 1: Clinical correlation of Theranos assay with R&D kit

Stability

Stability of the IGF-1 Theranos cartridges was evaluated at 4°C and room temperature. Figure 2 shows the responses for unspiked samples and samples spiked with three different calibrator levels. Stability at 4°C is established at up to 12 weeks (further study ongoing). Stability at room temperature is compromised after 2 weeks.

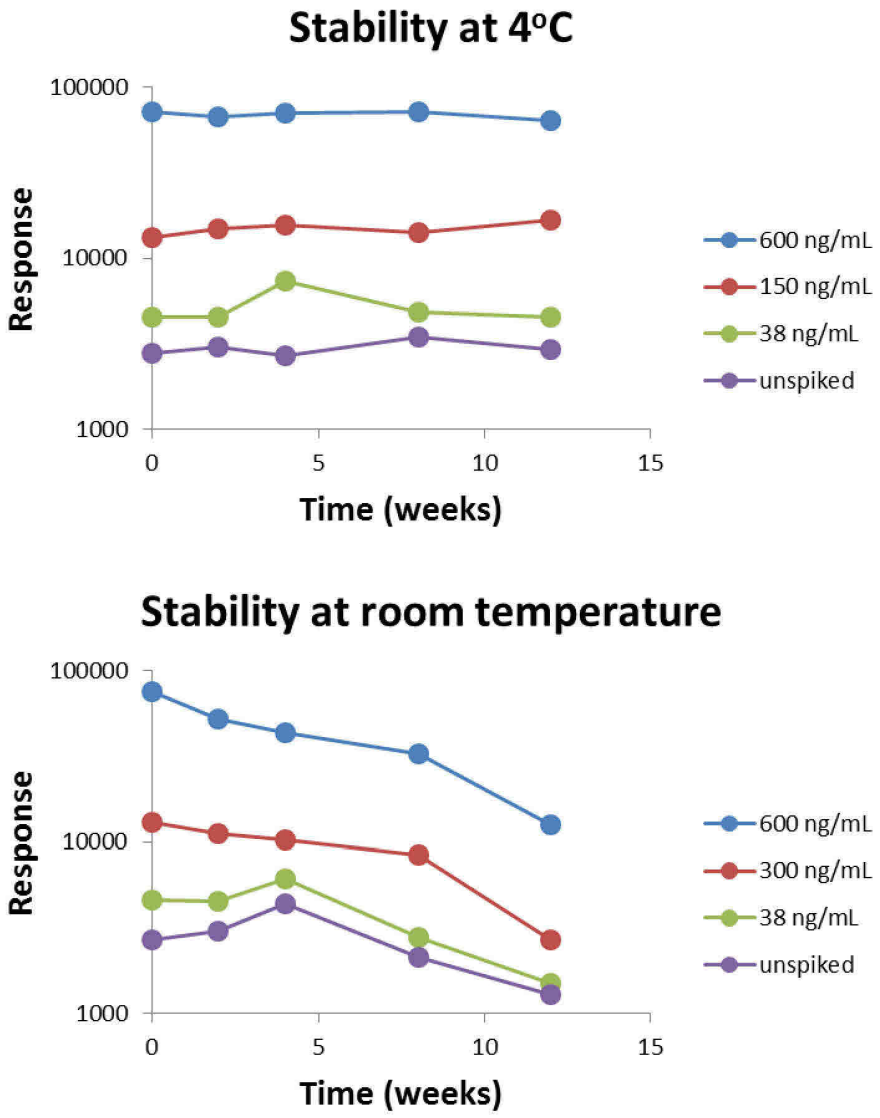


Figure 2: Stability study at 4°C and room temperature

Matrix effect

Table 8 shows the matrix effects for grossly hemolysed and lipemic serum samples. The results indicate lower recovery due to such gross levels of hemolysis and lipemia in the matrix causing interference in the assessment of IFG-1 analyte.

.

Table 8: Matrix effects on recovery

Hemolysed Serum

Calibrator conc. (ng/mL)	Calculated conc. (ng/mL)	% recovery
1200	1298.6	108
600	499.0	83.2
300	127.7	42.6
150	97.4	64.9
unspiked	0	-

Lipemic Serum

Calibrator conc. (ng/mL)	Calculated conc. (ng/mL)	% recovery
1200	878.6	73.2
600	361.2	60.2
300	136.1	45.4
150	67.2	44.8
unspiked	0	-

References

1. IGF-1 and IGFBP-1 and Cognitive Function in Older Men and Women. Wael K. Al-Delaimy, MD, PhD, Denise von Muhlen, PhD, and Elizabeth Barrett-Connor, MD. Department of Family and Preventive Medicine, School of Medicine, University of California, San Diego, La Jolla, California, USA.

2. Glycosaminoglycans inhibit formation of the 140 kDa insulin-like growth factor-binding protein complex. Robert C. BAXTER. Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, N.S.W. 2050, Australia

3. Serum Insulin-Like Growth Factor-1 Binding Proteins 1 and 2 and Mortality in Older Adults: The Health, Aging, and Body Composition Study. Donglei Hu, PhD, Ludmila Pawlikowska, PhD, Alka Kanaya, MD, Wen-Chi Hsueh, PhD, Lisa Colbert, PhD, Anne B. Newman, MD, Suzanne Satterfield, MD, Dr PH, Clifford Rosen, MD, Steven R. Cummings, MD, Tamara B. Harris, MD, and Elad Ziv, MD