SUN-0642

Development of IGF-1 ELISA Assays to Measure Free and Total Circulating IGF-1*

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INTRODUCTION

Insulin-like growth factor I (IGF-I, a.k.a. somatomedin C) is a 7.6 kDa, 70 amino acid residue peptide, which mediates the actions of growth hormone (GH). In vivo, IGF-I is secreted by the liver and several other tissues and is postulated to have mitogenic and metabolic actions at or near the sites of synthesis; i.e. paracrine effects. IGF-I also appears in the peripheral circulation where it circulates primarily in a high molecular weight tertiary complex with IGF-binding protein-3 (IGFBP-3) and acid-labile subunit (ALS). A smaller proportion of IGF-I circulates in association with other IGF- binding proteins.

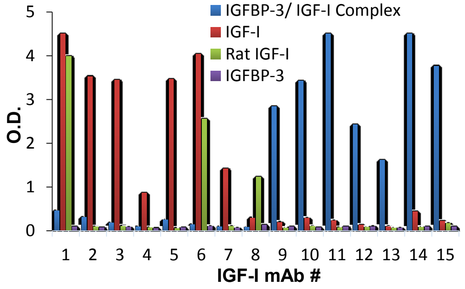
Recently, there has been research interest in the measurement of serum/plasma "unbound" IGF-I which, theoretically, is the biologically active fraction. Unbound IGF-I has also been observed in saliva. It is likely that the measured unbound IGF-I fraction is a combination of the true unbound and the fraction of IGF-I that can be readily dissociated from IGFBP's under the specific assay conditions. Previous methods such as size-exclusion chromatography and filtration have been used to estimate the unbound IGF-I fraction, however they have the theoretical disadvantage of altering the sample matrix and the equilibrium between IGF-I and IGFBP's. An assay that allows detection of unbound and total IGF-I using common calibrators is needed in the field. This will allow one to directly measure the ratio of total IGF-1 to dissociable fraction of IGF-I in an individual subject.

METHOD

Assay	mAb Pair	Sample	Calibrator range
Bioactive IGF-I	6 & 14	Neat	0.5 – 35ng/mL
Total IGF-I	6 & 14	Treated (1:25)	0.5 – 35ng/mL
Rat/Mouse Free IGF-I	1 & 15	Neat	0.25 – 10ng/mL
Rat/Mouse Total IGF-I	1 & 15	Treated (1:10)	0.5– 20ng/mL

The calibrators have been standardized to World Health Organization IGF-1 preparation NIBSC code 91/554, version 5.0.

Antibody Screening: Antibodies were screened on the IGF-1 related antigens and selected based on their specificity and sensitivity.

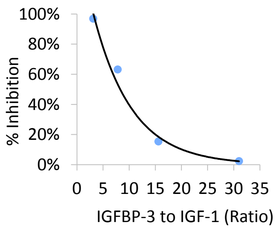


RESULTS

Limit of Detection (LoD): Calculated by the interpolation of mean plus two standard deviations of 20 replicates of calibrator A (0 ng/mL) and calibrator B.

Bioactive/ Total IGF-I	Rat/Mouse Free IGF-I	Rat/Mouse Total IGF-I
0.025ng/mL	0.11ng/mL	0.04ng/mL

Inhibition: Varying concentrations of IGFBP-3 were spiked into IGF-I and incubated for 30 minutes. IGF-I/IGFBP-3 complex formed was studied for inhibition in the Bioactive IGF-1 ELISA assay.



Cross Reactivity: Recombinant and native cross-reactants were spiked in calibrator A (matrix) at 1000 ng/mL and run as unknowns. % cross-reactivity was calculated by dividing the observed concentration by expected concentration x 100.

Cross-reactant 1000 (ng/mL)	% Cross-reactivity	
	Bioactive/Total IGF-I	Rat/ Mouse Total IGF-I
IGFBP-2	0.00	0.00
IGFBP-3	0.04	0.00
IGFBP-4	0.00	0.00
IGFBP-5	0.00	0.00
Rat IGF-I	3.16	100
IGF-I/IGFBP-3	0.42	13.5
IGF-II	0.00	0.00

Imprecision: Reproducibility of the IGF-I assays were determined using kit controls and serum pools. The study included six assays with samples in replicates.

Bioactive IGF-I

Sample	Mean Conc.	Within Run	Between Run	Total
ID (n)	(ng/mL)	%CV	%CV	%CV
QC3 (24)	1.7	3.9	5.1	6.4
QC5 (12)	4.8	3.8	3.5	5.2

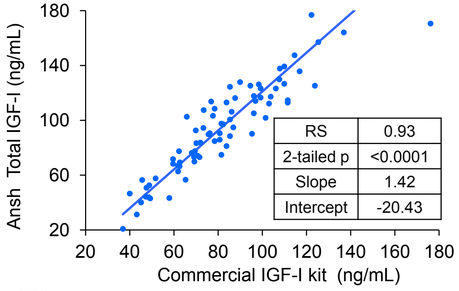
Total IGF-I

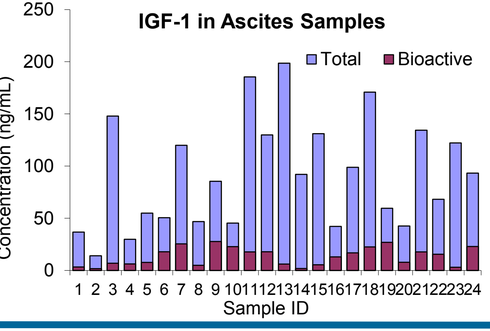
Sample	Mean conc.	Within Run	Between Run	Total
ID (n)	(ng/mL)	%CV	%CV	%CV
CI (24)	2.09	4.1	4.8	6.3
CII (23)	8.19	4.3	4.2	5.8
QC1 (24)	132.2	1.6	4.1	4.4

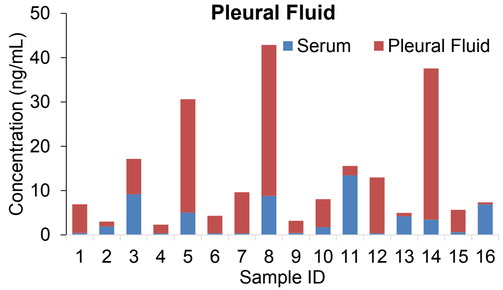
Interference: Interfering substances were spiked to 3 independent samples and % difference were calculated.

Interfering substance	Analyte (mg/mL)	Unspiked (ng/mL)	Spiked (ng/mL)	% Difference
Hemoglobin	1.35	1.03	1.09	4.92
		3.31	3.54	6.92
		2.70	2.84	5.26
Triglycerides	5	1.04	1.00	-3.57
		3.31	3.39	2.60
		2.70	2.84	4.96
Bilirubin	0.5	0.84	0.94	12.11
		3.17	3.44	8.75
		2.98	3.12	4.72

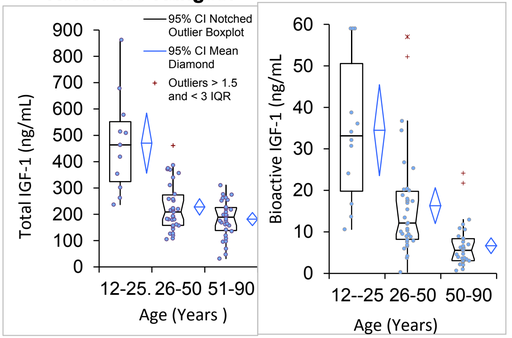
Method Comparison: Total IGF-I ELISA was compared to a commercial IGF-I ELISA kit using 77 serum samples.







Reference Ranges:



CONCLUSIONS

- Well characterized, highly sensitive, reliable and easy-to-perform IGF-1 assays have been developed to measure total and bioactive IGF-1 in serum, plasma, pleural fluid and ascites.
- These assays allow for a direct measurement of bioactive IGF-I to total IGF-I ratio for individual subjects using same calibrators and same mAb pair.
- The Rat/Mouse IGF-1 assays provide researchers an additional tool to study circulating IGF-1 in rodents.

*Research Use Only