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 unbound IGF-I fraction is a combination of the true unbound and the fraction of IGF-I that can readily dissociate from IGFBPs 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 IGFBPs. An assay that allows detection of unbound and total IGF-I using common calibrators is needed in the field. This will allow to directly measure the ratio of total IGF-I to dissociable fraction of IGF-I in an individual subject.

METHOD

RESULTS

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

Detection of IGF
IGFBP’s in vivo, which is of interest unbound IGF-I selected complex the binding fraction and 2.09 10ng/mL 0.5 %CV

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.

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

REFERENCES

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CONCLUSIONS

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