Validation of an ELISA Method for the Determination of Insulin-Like Growth Factor 1 (IGF-1) in Human Serum

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Introduction

Insulin-like growth factor 1 (**IGF-1**) is a protein is produced by the live and encoded by the *IGF1* gene. IGF-1 is similar in molecular structure to insulin and plays an important role in mediating growth hormone activity by inducing amino acid uptake, protein synthesis; and glucose utilization. Therefore, IGF-1 is essential to prenatal development, anabolic effect in adults, and metabolic control. IGF-I serum levels have been shown to increase from birth to puberty, followed by a decline through adulthood. IGF affects many primary physiological and pathological processes, including development, growth, metabolic regulation, tumorigenesis, atherosclerosis and angiogenesis[1].

IGF-1 was validated using a commercial ELISA kit[1] over a range of 31.25-1000 ng/mL. Assay parameters were evaluated and optimized to improve the performance for the method.

Materials and methods

Specific reagents used in the assay and procedure are summarized in Table 1 and Table 2. The assay quantitates IGF-1 in human serum by sandwich enzyme linked immunosorbent assay (ELISA). IGF-1 binds to plates coated with monoclonal capture antibody specific for IGF-1 and the detection is accomplished using a polyclonal antibody conjugated to horseradish peroxidase (HRP). Tetramethy-benzidine (TMB) is the substrate. The standards were prepared in rat serum and prior to preparing the QCs in human serum, it was necessary to screen the matrix in order to identify the matrices with below lower limit of quantitation (BQ) levels of endogenous analyte.

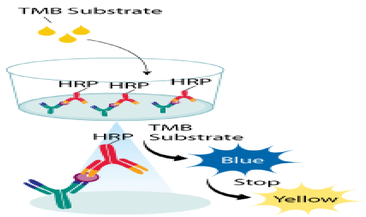

Figure 1. ELISA assay design

Reagents	Description
IGF-1 Microplate	96-well polystyrene microplate coated with mouse monoclonal antibody against IGF-1
IGF-1 Conjugate	Polyclonal antibody against IGF-1 conjugated to horseradish peroxidase
Assay diluent	Buffered protein base
Pretreatment A	Acid dissociation solution
Pretreatment B	Buffered protein
Color reagent A	Hydrogen Peroxide
Color reagent B	Stabilized chromgen (Tetramethylbenzidine)
Stop solution	2N Sulfuric Acid

Table 1. Assay reagent

Reagent	96-well coated plate		
	standards	Quality controls	Validation samples
Plate A			
Pretreatment A (µL)	190	190	190
Sample (µL)	10	10	10
Cover plate A and place on a plate shaker set at 650 rpm for 10 minutes			
Plate B			
Pretreatment B (µL)	80	80	80
Sample from plate A	20	20	20
Cover plate B and place on a plate shaker set at 650 rpm for 10 minutes			
Plate C			
Assay dilutent (µL)	150	150	150
Sample from plate C	50	50	50
Cover plate C and place at 4°C for 2 hours			
Wash plate 4x with wash buffer			
IGF-1 Conjugate (µL)	200		
Cover plate and place at 4°C for 1 hour			
Wash plate 4x with wash buffer			
Substrate (µL)	200		
Cover plate and incubate at room temperature for 30 minutes protected from light			
2N H_2SO_4 stop solution (µ	50		
Read the plate using reader at 540 nm subtracted from 450 nm			

Results

The validation parameters of accuracy, precision, robustness, selectivity, hemolysis, freeze-thaw stability, long-term stability, and bench-top stability were evaluated for each compound. The intra-assay and inter-assay (pooled) precision (%CV) and accuracy (%RE) for each validation sample concentration was ≤ 20% (≤ 25% for LLOQ and ULOQ) for the four compounds. The inter-assay total error (|%CV| + |%RE|) was < 30% (< 40% for LLOQ).

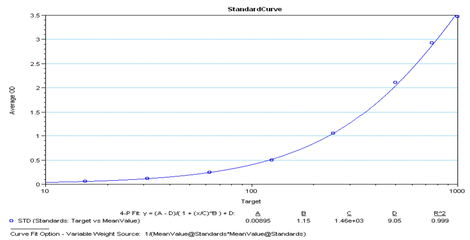

Figure 2. Representative calibration curve in rat serum

Characteristic	Statistic	Nominal concentration (ng/mL)				
		LLOQ	QCL	QCM	QCH	ULOQ
		31.3	87.5	359	685	993
# Results	N	35	36	36	36	35
Accuracy	Mean Bias (%RE)	5.5	-14.8	8.9	12.9	7.3
Precision	Interbatch (%CV)	5.3	5.3	5.6	5.3	4.9
Total Error	|Mean| + Interbatch	10.816	20.125	18.244	18.244	12.204

Table 3. Total Error for Precision and Accuracy

Sample	Unspiked Matrix (ng/mL)	Nominal concentration at QCM (ng/mL)	Actual concentration (ng/mL)	%RE	Nominal concentration at QCH (ng/mL)	Actual concentration (ng/mL)	%RE
1	119	417.215	374.104	10.30	715.430	611.860	14.50
2	230	526.550	470.540	10.60	823.100	759.330	7.75
3	157	454.645	423.207	6.91	752.290	696.605	7.40
4	42	341.666	338.221	1.01	641.031	604.795	5.65
5	148	445.780	471.006	-5.660	743.560	743.174	0.05
6	135	432.975	387.391	10.50	730.950	611.476	16.30
7	111	409.335	364.556	10.900	707.670	640.771	9.45
8	179	476.315	434.674	8.74	773.630	674.590	12.80
9	98	396.530	329.051	17.00	695.060	608.066	12.50
10	135	432.975	406.887	6.03	730.950	680.685	6.88

Table 4. Selectivity evaluation of IGF-1 in human serum

Validation Experiment	Result
Human serum	
Short Term Stability (Bench)	23 Hours
Short Term Stability (4°C)	23 Hours
Freeze Thaw Stability (-80°C); thawed at room temperature	6 Cycles
Freeze Thaw Stability (-80°C); thawed at 4°C	6 Cycles
Selectivity	No interference observed
Hemolysis	Not suitable
Dilution (fold)	10
Long-Term Freezer Stability (-80°C)	113 Days
Rat serum	
Short Term Stability (4°C)	3 Hours
Freeze Thaw Stability (-80°C)	3 Cycles
Long-Term Freezer Stability (-80°C)	113 Days

Table 5. Validation summary of IGF-1 in human serum

Conclusions

A sensitive assay for the detection of IGF-1 was developed, optimized, and validated over a range of 31.25-1000 ng/mL. The method were reliable and robust, and considered suitable for the analysis of IGF-1 in human serum.

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Literature cited

1. R&D Systems. Quantikine® ELISA-Human IGF-1 Immunoassay- (Product# SG100).

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