Validation of an ELISA Method for the Determination of Human Growth Hormone (hGH) in Human Serum

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Introduction

Growth hormone (hGH) is a single-chain polypeptide secreted by somatotropic cells and responsible for growth, cell reproduction and regeneration in humans and animals. Growth hormone production may occur directly or indirectly by stimulated production of IGF-1. Growth hormone is used in medicine to treat growth disorder and growth hormone deficiency. The overproduction of hGH can cause acromegaly, cardiovascular disease, and respiratory disease. Growth hormone deficiency can cause increased cardiovascular risk, insulin sensitivity, and decreased muscle mass. While legal, GH use as an anabolic agent by athletes has been controversial[1].

Growth hormone (hGH) was validated using a commercial ELISA kit[1] over a range of 0.300-45.0 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 hGH in human serum by sandwich enzyme linked immunosorbent assay (ELISA). Growth hormone (hGH) binds to plates coated with monoclonal capture antibody specific for GH and the detection is accomplished using a polyclonal antibody conjugated to horseradish peroxidase (HRP). Tetramethy-benzidine (TMB) is the substrate. Prior to preparing the standards and 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.

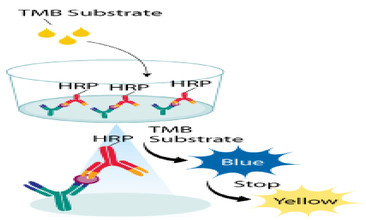

Figure 1. ELISA assay design

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

Table 1. Assay reagents

Reagent	96-well coated plate		
	standards	Quality controls	Validation samples
Calibrator Diluent (µL)	140	140	140
Sample (µL)	10	10	10
Cover plate and place on a plate shaker set at 600 rpm at 38°C for 3 hours			
Wash plate 4x with wash buffer			
GH Conjugate (µL)	200		
Cover plate and place at room temperature for 2 hour			
Wash plate 4x with wash buffer			
Substrate (µL)	200		
Cover plate and incubate at room temperature for 28 minutes protected from			
2N H_2SO_4 stop solution (µ	100		
Read the plate using reader at 450 nm and 570 nm			

Table 2. Assay procedure

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).

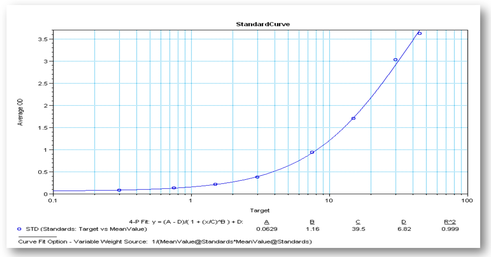

Figure 2. Representative calibration curve in human serum

Characteristic	Statistic	Nominal concentration (mIU/mL)				
		LLOQ 0.300	QCL 1.00	QCM 5.00	QCH 35.0	ULOQ 45.0
# Results	N	36	35	36	36	36
Accuracy	Mean Bias (%RE)	-1.7	4.3	2.5	7.0	-3.2
Precision	Interbatch (%CV)	23.1	16.6	12.4	8.1	3.4
Total Error	Mean| + Interbatch	24.797	20.821	14.946	15.083	6.583

Table 3. Total Error for Precision and Accuracy

Sample	Unspiked Matrix	Theoretical concentration	Matrix spiked at QCL 1.00 ng/mL	%RE
1	<LLOQ	1.00	1.09	9.00
2	0.519	1.52	1.58	3.95
3	1.02	2.02	2.18	7.92
4	<LLOQ	1.00	1.15	15.0
5	<LLOQ	1.00	1.15	15.0
6	<LLOQ	1.00	1.25	25.0
7	<LLOQ	1.00	1.14	14.0
8	<LLOQ	1.00	1.19	19.0
9	12.6	13.6	12.0	-11.8
10	<LLOQ	1.00	0.797	-20.3
LLOQ = 0.300 ng/mL				

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

Validation Experiment	Result
Short Term Stability (ambient)	22 Hours
Freeze Thaw Stability (-80°C)	4 Cycles
Freeze Thaw Stability (-20°C)	4 Cycles
Selectivity	No interference observed
Hemolysis	No interference observed
Dilution (fold)	100
Long-Term Freezer Stability (-80℃)	127 Days

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

Conclusions

A sensitive assay for the detection of hGH was developed, optimized, and validated over a range of 0.300-45.0 ng/mL. The method were reliable and robust, and considered suitable for the analysis of hGH in human serum.

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Literature cited

1. R&D Systems. Quantikine® ELISA-Human Growth Hormone Immunoassay- (Product# DGH00).

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