Validation of an ELISA Method for the Determination of Recombinant Human Granulocyte Colony Stimulating Factor (G-CSF) in Human Serum

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Introduction

Granulocyte Colony Stimulating Factor (G-CSF) stimulates the production of white blood cells. In oncology and hematology, a recombinant form of G-CSF is used with certain cancer patients to accelerate recovery from neutropenia after chemotherapy, allowing higher-intensity treatment regimens. Chemotherapy can cause myelosuppression and unacceptably low levels of white blood cells, making patients susceptible to infections and sepsis.

G-CSF was validated using a commercial ELISA kit[1] over a range of 70-5000 pg/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. A Nunc plate was coated with mouse anti-human G-CSF and incubated overnight. Standards, quality control samples, and human sera samples were diluted in BSA/PBS and added to the coated and blocked Nunc plate (1%; 1:5; 100 µL). After incubation for 2 hours at room temperature, the plate was washed and detection antibody (biotinylated goat anti-human G-CSF) was added (100 µL). Then, the plate was incubated at room temperature for 2 hours, washed and a streptavidin HRP (100 µL) was added and allowed to incubate for 20 minutes. The plate was washed and TMB substrate was added (100 µL) and incubated for 10 minutes after which sulfuric acid was added (2N; 50 µL) and the plate was read at 450nm. Prior to preparing the standards and QCs in human serum, it was necessary to screen the matrix to identify the matrices with below lower limit of quantitation (BQ) levels of endogenous analyte.

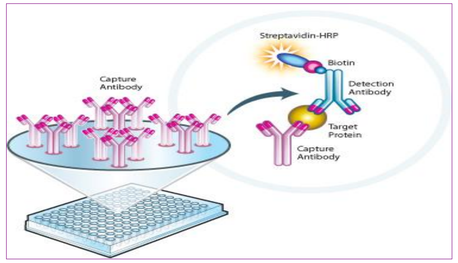

Figure 1. G-CSF ELISA Assay Design

Reagents	Description
G-CSF	Human rDNA derived
Capture Antibody	Mouse anti-human G-CSF
Detection Antibody	Biotinylated goat anti-human G-CSF
Streptavidin-HRP	Streptavidin conjugated to horseradish-peroxidase
Stop solution	2N Sulfuric Acid

Table 1. Assay Reagents

Results

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

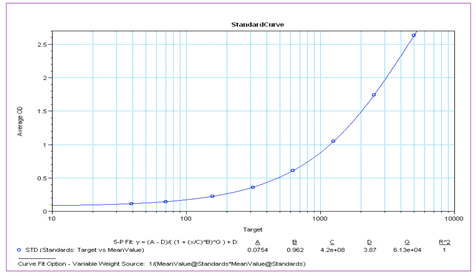

Figure 1. Representative Calibration Curve

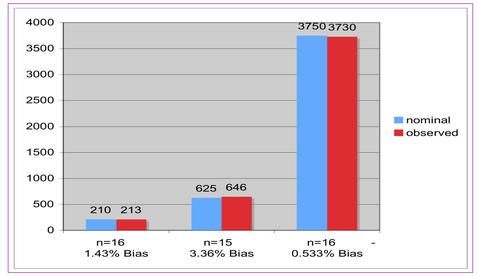

Chart 1. Performance of Run Qualifiers

Characteristic	Statistic	Nominal concentration (mIU/mL)				
		LLOQ	QCL	QCM	QCH	ULOQ
		70.0	210.0	625	3750	5000
# Results	N	34	36	36	36	36
Accuracy	Mean Bias (%RE)	3.5	1.5	3.2	0.1	-3.1
Precision	Interbatch (%CV)	13.4	7.3	5.8	4.0	7.0
Total Error	|Mean| + Interbatch	16.892	8.708	9.054	4.077	10.143

Table 2. Total Error for Precision and Accuracy

Sample	Unspiked Matrix	Matrix spiked at QCL 210 pg/mL	%RE	Matrix spiked at QCH 3750 pg/mL	%RE
1	<LLOQ	222	5.71	4000	6.67
2	<LLOQ	208	-0.952	3660	-2.40
3	<LLOQ	204	-2.86	4010	6.93
4	<LLOQ	174	-17.1	3240	-13.6
5	<LLOQ	190	-9.52	3720	-0.800
6	<LLOQ	193.0	-8.10	3230	-13.9
7	<LLOQ	181	-13.8	3410	-9.07
8	<LLOQ	188	-10.5	3590	-4.27
9	<LLOQ	215.0	2.38	3780	0.800
10	<LLOQ	209	-0.476	3670	-2.13

LLOQ = 70.0 pg/mL

Table 3. Selectivity Evaluation of G-CSF in Human Serum

(pg/mL)		%RE
Nominal	Concentration	
2500	2450	-1.87
1000	931	-6.87
500	478	-4.33

Dilution of sample prepared from high stock was shown to be linear down to 500 pg/mL

Table 4. Dilution Linearity of G-CSF in Human Serum

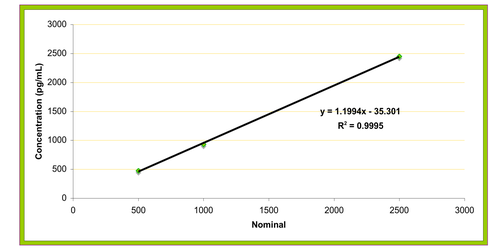

Figure 3. Dilution Linearity of G-CSF in Human Serum

Validation Experiment	Result
Short Term Stability (Bench)	27 Hours
Freeze Thaw Stability (-70°C)	4 Cycles
Freeze Thaw Stability (-20°C)	2 Cycles
Selectivity	No interference observed
Dilution (fold)	100
Hemolysis	No discernable effect
Long-Term Freezer Stability (-70°C)	104 Days
Long-Term Freezer Stability (-20°C)	104 Days
Reference Standard stability	134 Days

Table 5. Validation Summary of G-CSF in Human Serum

Conclusions

A sensitive assay for the detection of G-CSF was developed, optimized, and validated over a range of 70-5000 pg/mL. The method were reliable and robust, and considered suitable for the analysis of G-CSF in human serum.

Literature cited

R&D Systems. Human G-CSF DuoSet ELISA kit- (Product# DY214E).

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