Poster Board No. T2070

Validation of a Highly Sensitive Immunoassay for the Quantitation of Interferon Beta in Autoimmune Sera

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Abstract

Purpose
The role of interferons (IFN) in autoimmune disease is crucial to understanding the etiology and treatment of these diseases.

A highly sensitive immunoassay was validated and utilized for the quantitation of interferon beta in autoimmune sera.

Methods
VeriKine-HS™ Human Interferon Beta Serum ELISA kit was validated in 100% sample matrix. The lower limit of quantitation (LLOQ) was determined using independent spikes in pooled normal sera. Quantitation of endogenous levels in normal human and autoimmune patient sera as well as spike recovery were performed. Specificity against IFN Alpha, IFN Gamma and IFN Omega was assessed up to 10 ng/mL and assay response in the presence of hemolysis examined. Finally, multiple sclerosis (MS) samples measured across two sites and different lots of kits were correlated.

Results
The method was validated with an analytical range of 2.34 – 150 pg/mL and showed dilutional linearity up to 1:2187. Interassay precision (CV) was ≤13.1% with a mean % bias 17.1. The LLOQ of the assay was determined to be 2.34 pg/mL. No IFN Beta was detected in normal sera. Median quantifiable IFN Beta levels were 17.3 pg/ml in MS sera (n = 47), with 26 quantifiable sera and 21 below LLOQ. One hundred percent (100%) specificity was demonstrated against IFN Alpha, IFN Gamma and IFN Omega in both blank matrix and matrix spiked with 60 pg/mL IFN Beta. Whereas no interference was detected up to 120 pg/mL IFN Beta at 10% hemolysis, a 23.2% bias at 100% hemolysis was observed at 120 pg/mL. MS samples showed excellent correlation (r^2=0.9975) which is indicative of a robust and reproducible assay.

Conclusion
A precise and accurate method was validated for measuring IFN Beta. These evaluations confirm that this highly sensitive immunoassay is suitable for evaluation of autoimmune sera.

Introduction

Interferons are low molecular weight proteins that belong to the class of glycoproteins known as cytokines. Interferons have been identified as important immuno-modulators in autoimmune diseases.

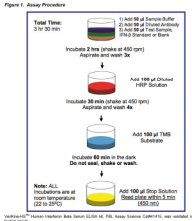
IFN-Beta is the most accepted bio-therapeutic for the treatment of multiple sclerosis (MS) and has shown to decrease relapses, brain lesions, and slow neuro-degeneration in patients [1]. However, the clinical response to IFN-Beta is highly variable [2]. Hence, understanding the mechanism of action of IFN-Beta in MS treatment may prove to be highly valuable in improving the efficacy of this therapy.

The association between type I IFN and Systemic lupus erythematosus (SLE) is the subject of intense investigation (3). An IFN response signature, associated with increased expression of IFN and IFN-stimulated genes, has been identified in SLE patients (4,5). While much of the focus has been on IFN Alpha, the role of IFN Beta is not well understood.

Having tools to accurately measure IFN Beta in complex matrices such as MS and SLE sera is imperative in understanding the connection between IFN response signatures and etiology of these diseases.

Method

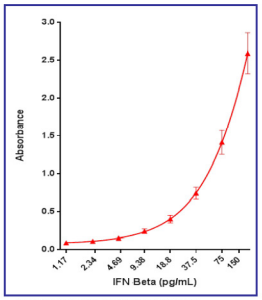
Figure 1. Assay Procedure



VeriKine-HS™ Human Interferon Beta Serum ELISA kit, PBL Assay Science Cat#41410, was validated in 100% human serum.

Results

Figure 2. Standard Curve



The method was validated with an analytical range of 2.34 -150 pg/mL.
The graph display represents mean data from 15 runs.

Table 1. Accuracy and Performance

	Accuracy and Precision			
		InterAssay Performance		
	QCs pg/mL	Mean Recovery pg/mL	%Bias	Precision %CV
LLOQ	2.34	1.9	13.1	-17.1
LQC	6.5	5.9	-9.8	9.6
MQC	60	51.5	-14.1	4.7
HQC	120	101	-16.1	4.18
ULOQ	150	132	-11.9	4.39

Accuracy and precision was demonstrated by successful completion of six (6) independent accuracy and precision runs performed over four days by two analysts.

Mean calculated values for each of the validation samples in every A&P run was ≤13.6% of their respective nominal concentrations (data not shown).

Overall mean accuracy and precision for each of the QCs levels is ≤16.1% Bias and ≤17.1 % CV.

Table 2. Selectivity - Normal Individual

	Spiked at 5.0 pg/mL IFN Beta			
Individual Normal Serums	Blank pg/mL	Observed pg/mL	% CV	% Bias
BRH601306	BLOQ	4.75	3.0	-5.0
BRH601308	BLOQ	5.03	5.1	0.6
BRH601309	BLOQ	4.85	4.5	-3.0
BRH601314	BLOQ	3.58	21.3	-28.4
BRH601316	BLOQ	5.09	4.3	1.8
BRH601323	BLOQ	5.16	1.5	3.2
BRH601324	BLOQ	4.85	0.6	-3.0
BRH601325	BLOQ	4.78	1.9	-4.4
BRH601326	BLOQ	4.69	3.9	-6.2
BRH601340	BLOQ	4.64	1.2	-7.2

Ten individual samples were tested unspiked and spiked at 5.0 pg/mL. Ten unspiked individual blank samples measured below LLOQ, nine spiked samples measured within 20% of nominal.

Table 3. Selectivity - SLE patients

	Spiked at 6.5 pg/mL IFN Beta			
Individual SLE Serums	Blank pg/mL	Observed pg/mL	% CV	% Bias
SLEsp1	BLOQ	6.58	4.41	1.23
SLEsp10	BLOQ	6.29	1.24	-3.23
SLEsp11	BLOQ	6.52	4.98	0.31
SLEsp12	BLOQ	6.30	3.7	-3.08
SLEsp13	BLOQ	6.69	3.06	2.92
SLEsp14	BLOQ	6.86	1.34	5.54
SLEsp15	BLOQ	6.24	0.91	-4.00
SLEsp16	BLOQ	6.22	2.38	-4.31
SLEsp2	BLOQ	6.63	0.53	2.00
SLEsp3	BLOQ	6.36	0.56	-2.15
SLEsp4	BLOQ	6.33	1.12	-2.62
SLEsp5	BLOQ	6.43	0.88	-1.08
SLEsp6	BLOQ	7.16	1.38	10.2
SLEsp7	BLOQ	6.62	1.5	1.85
SLEsp8	BLOQ	5.87	1.45	-9.69
SLEsp9	BLOQ	6.50	0	0

SLE blank samples measured below LLOQ both at Smithers Avanza and PBL Assay Science. All 16 samples spiked at 6.50 pg/mL measured within 20% of nominal.

Table 4. Hemolysis

	LQC 6.5 pg/mL IFN Beta		MQC 60.0 pg/mL IFN Beta		HQC 120 pg/mL IFN Beta	
	Mean Recovery	%Bias	Mean Recovery	%Bias	Mean Recovery	%Bias
10% hemolysis	6.39	-1.7	60.6	1.0	116	-3.3
100% hemolysis	5.85	-10	48.9	-18.5	92.2	-23.2

The effect of hemolysis on individual samples was examined by spiking 10% and 100% hemolyzed serum at the low, mid and high QC concentrations and determine IFN Beta recovery. Ten (10) % hemolysis had no effect on assay response at 6.5, 60.0 and 120 pg/mL of IFN Beta.

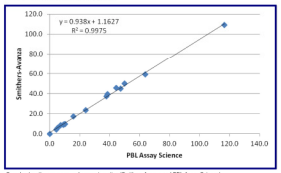
100% hemolysis had no effect on assay response at 6.5 and 60.0 pg/mL of IFN Beta. At 120 pg/mL of IFN Beta there was a 23.2% bias in 100% hemolyzed serum.

Table 5. Specificity

	Pooled Serum 0 pg/mL IFN Beta		MQC 60.0 pg/mL IFN Beta	
	pg/mL	%bias	pg/mL	%bias
1.0 ng/mL IFN Alpha	BLOQ	NA	55.6	-7.3
10 ng/mL IFN Alpha	BLOQ	NA	57.3	-4.5
1.0 ng/mL IFN Gamma	BLOQ	NA	55.3	-7.8
10 ng/mL IFN Gamma	BLOQ	NA	59.4	-1.0
1.0 ng/mL IFN Omega	BLOQ	NA	53.2	-11.3
10 ng/mL IFN Omega	BLOQ	NA	52.9	-11.8

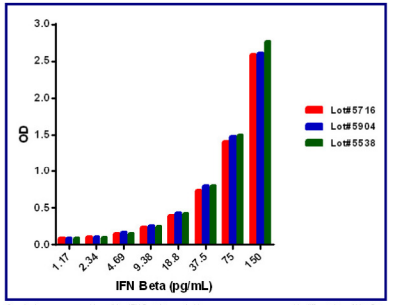
Assay is specific for IFN Beta as IFN Alpha, IFN Gamma, and IFN Omega had no effect on recovery of IFN Beta; all results were within ±20% of the target.

Figure 3. Correlation of MS Samples



Samples (read) were measured across two sites (Smithers Avanza and PBL Assay Science).
Median quantifiable IFN Beta levels were 17.3 pg/ml in MS sera (n = 47), with 26 quantifiable sera and 21 below LLOQ.
MS samples showed excellent correlation (r^2=0.9975) which is indicative of a robust and reproducible assay.

Figure 4. Reproducibility – Kit Lot Variability



Standard curves prepared by spiking IFN Beta into pooled human serum were compared in different lots of kits. Data obtained across three different lots are comparable which is indicative of a reproducible assay.

Table 6. Stability

Storage Condition	Bench Top	Refrigerated	Freeze/Thaw	-75°C
LQC, HQC Samples	3 hrs	3 hrs	6 Cycles	180 days*

*Ongoing
Stability was demonstrated for QCs stored under benchtop, 2-8°C, frozen and free-thaw conditions.

Conclusion

Interferons are important in drug therapy for many diseases involving the immune system. A precise and accurate method was validated for measuring IFN Beta and this highly sensitive immunoassay is suitable for the evaluation of autoimmune sera. The mechanism of action by which interferons work is complex and our understanding of the role of interferons will make a substantial impact on how diseases will be treated in the future.

References

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