P046

Development of a Novel Assay for Ultrasensitive Measurement of Serum Interferon-gamma

Sarah E. Wheeler, Jessica Suschak, Anahit Aghvanyan, Gunjan Tiwari, Anu Mathew, Martin Stengelin, and Jacob N. Wohlstadter

Meso Scale Discovery, Rockville, Maryland, USA

1 Abstract

Introduction: Interferon-gamma (IFN-γ) is a cytokine that is critical to innate and adaptive immunity. IFN-γ binds as a dimer to the IFN-γ receptor 1 and is involved in activation of Th1 cells, monocytes, and macrophages. This cytokine also up-regulates antigen presentation molecules and promotes immunoglobulin class switching in B cells. IFN-γ can induce both pro- and anti-inflammatory responses and is involved in many autoimmune disorders. Increased detection sensitivity of IFN-γ will allow for improved understanding of disease mechanisms and is necessary to better understand this cytokine as a biomarker and therapeutic target.

Methods: An ultrasensitive electrochemiluminescence assay format, S-PLEX™, based on MSD's MULTI-ARRAY® technology, was developed for IFN-γ and its performance was characterized. Monoclonal antibodies were evaluated and selected based on sensitivity, specificity, affinity, and performance characteristics against a WHO anchored IFN-γ calibrator.

Results: The detection limit for this novel IFN-γ immunoassay is 10 fg/mL, ~1,000 fold more sensitive than current IFN-γ immunoassays. The lower and upper limits of quantitation are 29 fg/mL and 50,400 fg/mL respectively. Typical intra-plate coefficients of variation (CVs) ranged from 6 to 14%. Inter-plate CVs were 15%, 9%, and 9% for low, mid, and high Quality Control (QC) sample levels, respectively (n=16 plates per QC level). The average percent recovery from spike experiments was 105% (n=12 specimens) and dilution linearity average recovery was 103% (n=12 specimens). The assay was optimized to minimize serum and plasma matrix effects and interferences. Specificity of the assay was demonstrated by analyte depletion using several anti-IFN-γ specific antibodies, indicating the assay is specific for the IFN-γ homodimer.

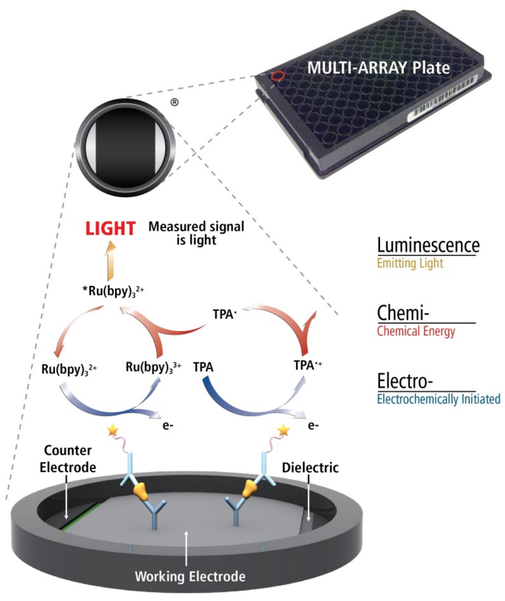
IFN-γ was detectable in serum and plasma of all apparently healthy specimens (n = 55) with a median value of 315 fg/mL in serum (n=31), 429 fg/mL in EDTA plasma (n=10), and 428 fg/mL in heparin plasma (n=14). IFN-γ did not appear to be elevated in a small number of rheumatoid arthritis specimens tested, but appeared to be elevated in some systemic lupus erythematosus and Crohn's specimens.

Conclusion: MSD developed an ultrasensitive IFN-γ immunoassay that is 1,000 times more sensitive than the current limits of IFN-γ immunoassays. MSD's S-PLEX immunoassay will aid in improved characterization of IFN-γ in disease states and in development of more targeted therapeutic interventions.

2 Methods

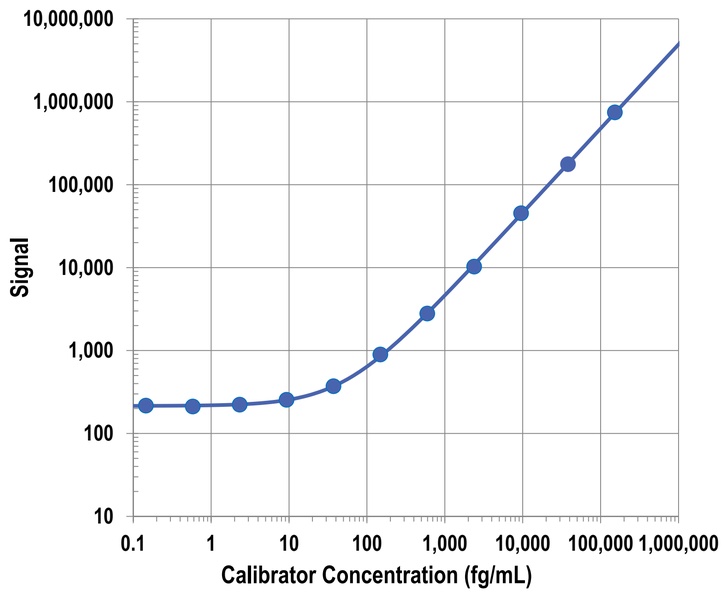
Electrochemiluminescence Technology

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY and MULTI-SPOT® microplates. We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY technology with significantly higher sensitivity.



3 Representative Calibrator Curve

The extended calibration curve is representative. Limit of detection (LOD) was typically below 10.0 fg/mL.



4 Lower Limit of Quantitation (LLOQ)

Lower limit of quantitation was determined using *E. coli* expressed recombinant human IFN-γ. Testing included five replicates per plate, two plates per day per operator (two operators) and was performed over four days (n=16 plates). The LLOQ was determined to be 29 fg/mL; intra-plate and inter-plate concentration %CVs were < 20%.

Expected Concentration (fg/mL)	Average Measured Concentration (fg/mL)	Accuracy (Average % Recovery)	Intra-plate Concentration (%CV)	Inter-plate Concentration (%CV)
56,000	58,528	105	6.9	10.0
39,200	40,624	104	5.6	9.1
1,944	1,893	97	5.8	9.3
115.0	115	100	8.1	15.0
57.5	56	97	10.8	16.9
28.8	29	100	14.0	20.0
14.4	14	96	19.7	29.6
Specification Range		70-130	< 20	<20

Limit of Detection (fg/mL)	4.9
Lower Limit of Quantitation (fg/mL)	29

5 Spike Recovery

Four serum, EDTA plasma, and heparin plasma samples were spiked with three concentrations of recombinant IFN-γ. Results are shown below.

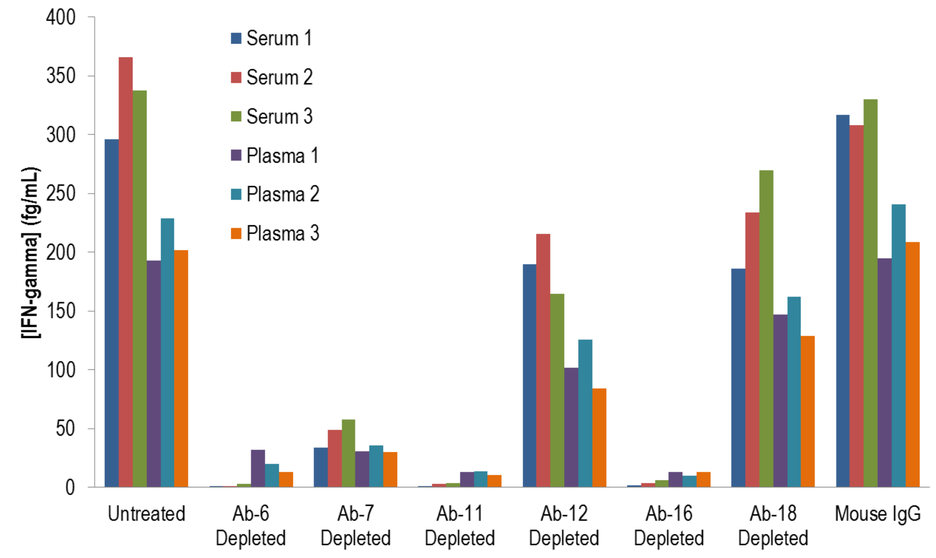
	Serum		EDTA Plasma		Heparin Plasma	
Spike Level (fg/mL)	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
10,000	109	101-120	104	89-116	118	114-123
5,000	102	92-112	99	96-103	107	101-114
1,000	100	97-105	102	98-105	106	101-110

6 Dilution Linearity

Four serum, EDTA plasma, and heparin plasma specimens were used to determine linearity from 2-, 4-, and 8-fold dilutions. Results are shown below.

	Serum		EDTA Plasma		Heparin Plasma	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
2	99	92-105	101	94-109	97	90-104
4	106	91-111	105	92-118	103	94-118
8	105	94-122	106	95-118	110	91-143

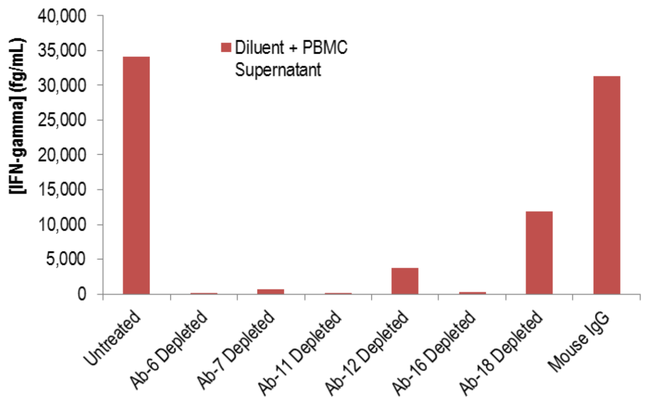
7 Depletion Study: Assay Specificity Demonstrated



Depletion Study

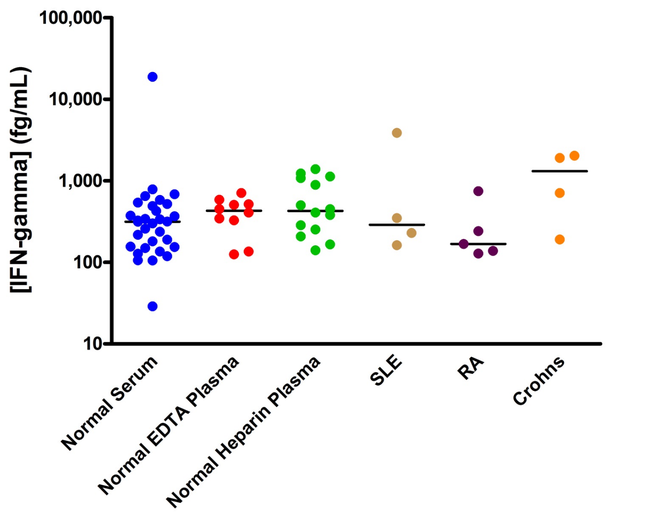
Specificity of the IFN-γ assay was demonstrated with a depletion study. Six anti-IFN-γ antibodies and control antibody (mouse IgG) were conjugated to magnetic beads and used to deplete IFN-γ from three serum and three plasma samples.

With the exception of Antibodies-12 and -18, all of the antibodies used were able to deplete IFN-γ. Antibodies-12 and -18 also showed poor depletion of 24-hour LPS-stimulated peripheral blood mononuclear cell (PBMC) supernatant spiked into diluent (at right). IFN-γ is known to be elevated in LPS-stimulated PBMC supernatant; measurement of high levels of IFN-γ in the supernatant provides further evidence of assay specificity. These results support that the analyte measured by the IFN-γ assay is the same analyte recognized by commercially available antibodies.



8 Sample Screening

Samples from 55 apparently healthy donors, 31 serum, 10 EDTA plasma, and 14 heparin plasma samples were tested. 100% of the samples had detectable levels of IFN-γ, with median levels of 315 fg/mL, 429 fg/mL, and 428 fg/mL for serum, EDTA plasma, and heparin plasma respectively. A limited number of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Crohn's specimens were tested.



9 Conclusion

We have demonstrated the performance of an ultrasensitive IFN-γ assay, with an LLOQ of 29 fg/mL (< 20%CV) and an LOD of 4.9 fg/mL. The assay demonstrated an average spike recovery of 105% and linearity of 103%. The S-PLEX IFN-γ assay specificity was verified by depletion studies with serum and plasma samples and detection of elevated levels in the established model of LPS-stimulated PBMCs. Furthermore, the S-PLEX assay format was able to quantify IFN-γ in 100% of the samples screened. MSD's S-PLEX immunoassay improves the characterization of IFN-γ in disease states and advances the development of targeted therapies.

Summary of Assay Performance	
Limit of Detection	4.9 fg/mL
Estimated Lower Limit of Quantitation	29 fg/mL
Median Normal Serum concentration (n=31)	315 fg/mL
Median Normal EDTA Plasma concentration (n=10)	429 fg/mL
Median Normal heparin Plasma concentration (n=13)	428 fg/mL
Percentage of samples within assay range (n=55)	100%

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