Measuring total IFNα at fg/mL concentrations in human blood

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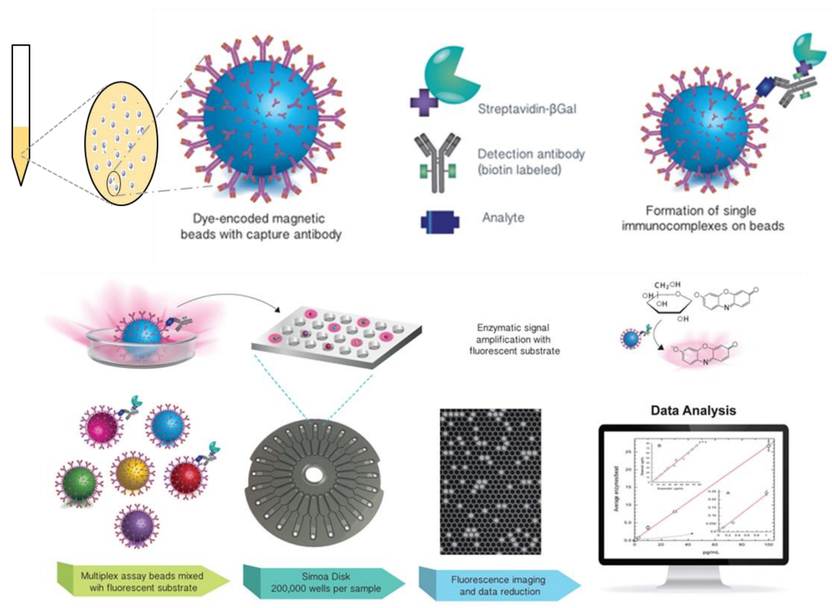
Abstract

Total IFNα has been challenging to quantify in normal human plasma and serum due to its low abundance and variety of subtypes. Here we describe the development of a digital immunoassay capable of measuring all 12 subtypes of human IFNα in plasma and serum using Quanterix's Simoa technology. The Simoa digital ELISA technology counts signal generated from single immunocomplexes formed on superparamagnetic beads confined in arrays of femtoliter-sized wells in which fluorescent molecules are highly concentrated.

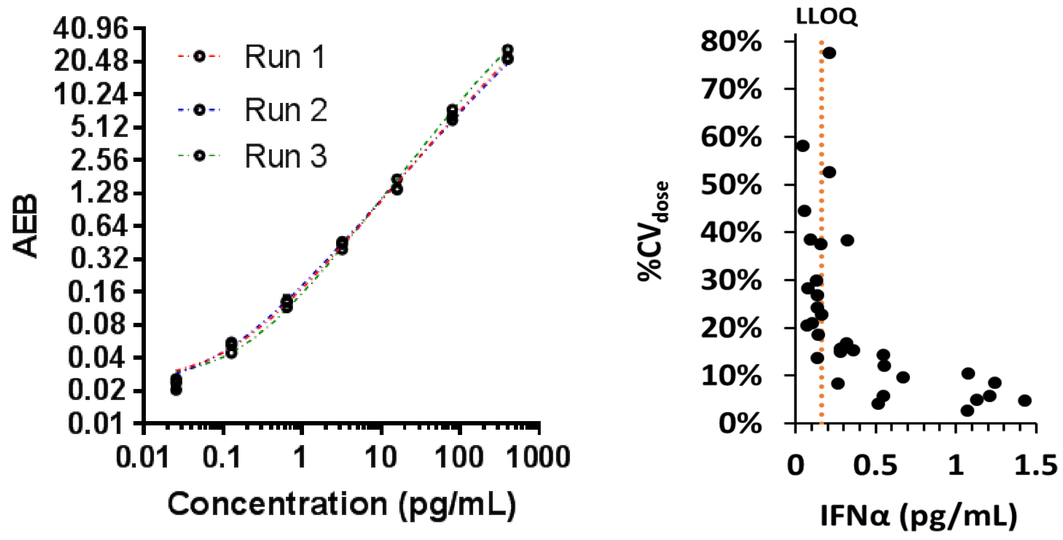
The Simoa IFNα multi-subtype assay has a dynamic range of approximately 3.5 orders of magnitude and an analytical sensitivity below 100 fg/mL. The median LOD (2.5 SD) over 12 runs was approximately 30 fg/mL and the LLOQ was approximately 80 fg/mL. The functional LLOQ, which accounts for sample dilution is 2x this value or approximately 160 fg/mL. This represents a significant improvement over commercial ELISAs, which claim IFNα sensitivities in the pg/mL range.

In repeated screens of 30 normal samples, up to 60% measured above LOD and testing of serum from Lupus patients and stimulated PBMCs resulted in concentrations within the dynamic range of the assay. Depletion experiments conducted by incubating unlabeled capture or detection antibody with these same samples demonstrated that the assay is specific for IFNα. IFNα has been useful in several applications including as a therapeutic agent, as a biomarker for monitoring response to immunotherapy, and as a marker of disease progression in human clinical and preclinical studies. In each example, increased sensitivity for detection of all subtypes of IFNα provides researchers and clinicians with the ability to measure the biomarker in both normal and acute samples with robustness required to advance precision medicine programs



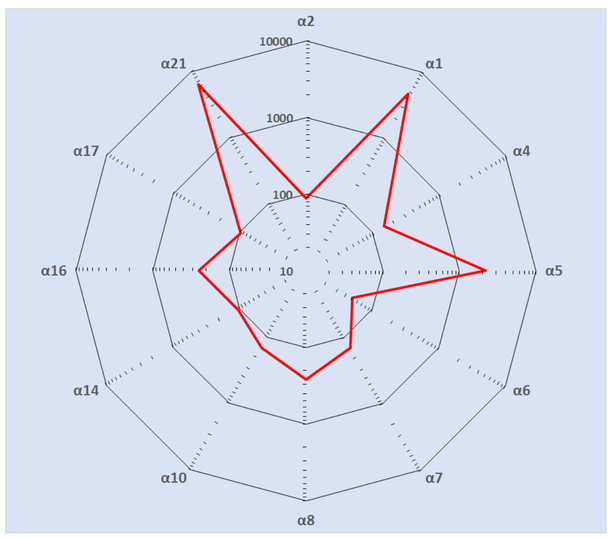
Results

Representative dose response of Simoa Human IFNα2 over a 4 log range. Three runs are shown (A). Each data point represents the mean of 2 replicates. Four-parameter curve fit is depicted. Mean Limit of detection (2.5 SD) over 12 individual runs is 36.3 fg/mL.



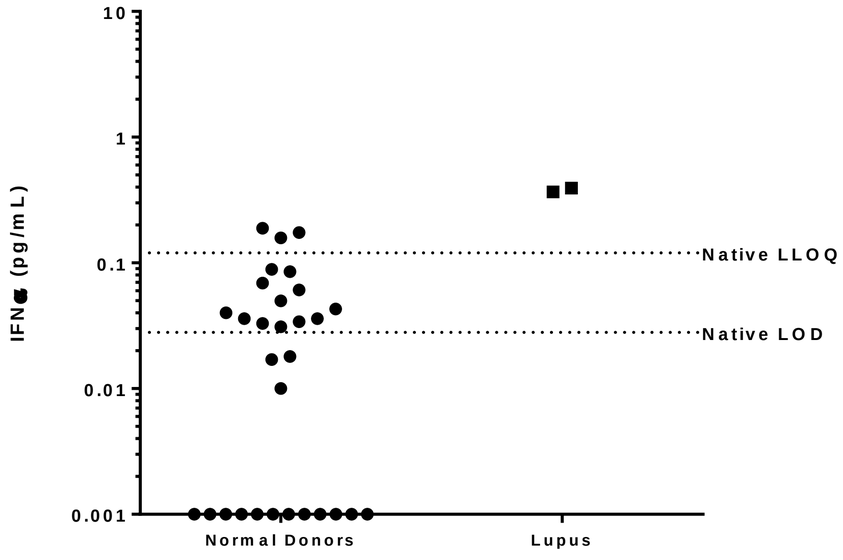
LLOQ evaluated using CV profiling and 20% CV. LLOQ was calculated using CV profiling, which employs a fixed signal CV (in the case of Simoa, AEB and Poisson CV) to estimate the associated concentration CV. The LLOQ is the concentration at which the low end of the calibration curve intersects the CV curve. The mean Lower Limit of Quantification over 12 runs is 80.2 fg/mL using the calibration curve, which translates to 160 fg/mL when accounting for 2x sample dilution. Two serum samples, two EDTA plasma samples, and two heparin plasma samples were spiked with 3 pg/mL of IFN α2 and serially diluted 2x in negative serum to a 64x dilution. Each sample dilution was diluted 1:1 in sample diluent and backfit to an in-run calibration curve run in duplicate (B).

LLOQ of individual IFNα subtypes. The mean functional Lower Limit of Quantification of each IFNα subtype was determined using CV profiling on calibration curves composed of individual IFNα subtypes. Results are shown in fg/mL.

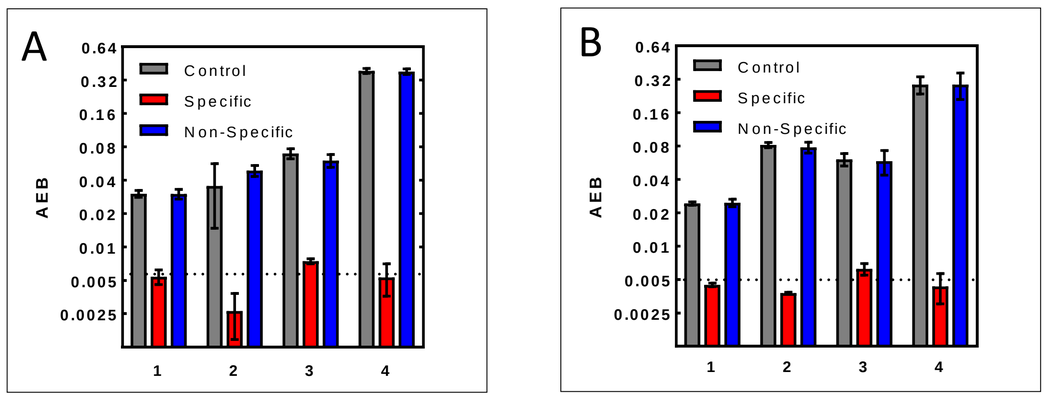


Subtype	LLOQ
α2	88
α1	4640
α4	148
α5	2180
α6	50
α7	146
α8	262
α10	144
α14	106
α16	248
α17	96
α21	6500

Measurement of IFNα in normal donor and lupus serum samples. 30 serum samples from normal donors and 2 serum samples from Lupus patients were read in triplicate in two additional runs. Concentrations were obtained by fitting to a calibration curve run in duplicate in the same run. Lupus sample concentrations represent the average concentration over both runs. CV profiling was used to estimate the LLOQ using the in-run calibration curve. 25 of the 30 samples (83%) produced AEB values above the background (calibrator diluent). 17 of these samples (57%) produced AEB values within the detectable range.



Measurement and Specificity of four IFNα positive samples. Two lupus samples (samples 1 and 2), one stimulated peripheral blood mononuclear cells (PBMC) sample (sample 3) and one serum sample spiked with 5 pg/mL of IFNα2 (sample 4) were pre-incubated with either 500X IFN α or IFNβ (control) detector antibody (A) or 100x IFNα or IFNβ (control) capture beads (B) for a period of 3 hours at room temperature before measurement in the assay.



Conclusions

The Simoa IFNα multi-subtype assay was optimized to detect all 12 subtypes of IFNα, minimize LOD/LLOQ, and maximize sample readability. LLOQ estimates using CV profiling with the calibration curve and sample dilution in the current assay configuration predict analytical LLOQ values of approximately 80 fg/mL and functional LLOQ (2X) values of approximately 160 fg/mL. Most normal serum and EDTA plasma samples could be read above assay background with significant numbers in the detectable range of the assay. The assay can measure stimulated IFNα levels in PBMCs and patient samples (Lupus) within the quantifiable range.