Simoa™ HD-1: a fully automated digital immunoassay analyzer capable of single molecule counting, sub-femtomolar sensitivity, and multiplexing

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INTRODUCTION

The aim of this work was to develop the next generation immunoassay analyzer capable of several orders of magnitude greater sensitivity than current best-in-class conventional immunoassay systems. The technology utilizes single molecule array (Simoa™) technology to usher in fully automated digital immunoassay and multiplexing capability to the clinical laboratory. Simoa technology isolates individual paramagnetic beads in arrays of femtoliter-sized wells and detects single enzyme-labeled proteins on these beads using sequential fluid flows in microfabricated polymer array assemblies for ultra-sensitive signal measurements. These array assemblies have been incorporated into a low-cost disk consumable. The array approach for assay signal quantification allows for rapid digital data acquisition and high throughput, enabling development of a fully automated system for low-cost measurement of clinically relevant biomarkers with high precision and unprecedented sensitivity across a broad dynamic range.

METHODOLOGY

Detection of single molecules using Simoa has been reported previously. In brief, proteins are captured on antibody-coated paramagnetic microbeads (2.7-μm diameter) and labeled with single enzymes, followed by partitioning single beads into arrays of femtoliter-sized wells and sealing the arrays in the presence of a fluorogenic substrate. We developed a low cost disk consumable that enables standard fluidics handling instrumentation to load and seal assay beads into the arrays using only fluidic flow. Beads with single enzyme label molecules are isolated in single wells in the presence of a substrate, and fluorescent product is allowed to build up within the 40 femtoliter confines of the wells. Hundreds to many thousands of single molecule signals are counted simultaneously

Fig. 1. Loading, sealing, and imaging of single paramagnetic beads in arrays of femtoliter-sized wells.
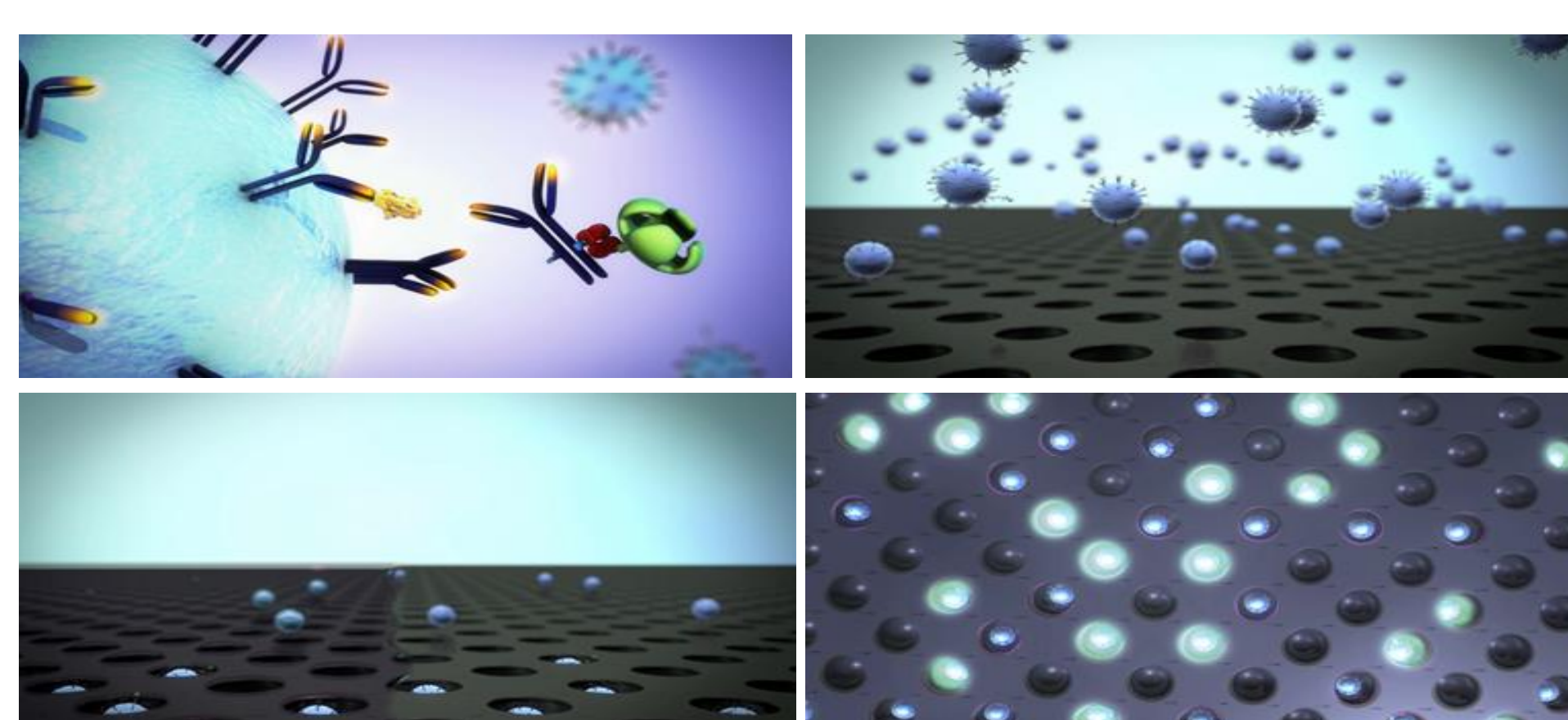
using a fluorescence microscope optical system and image analysis software.

Next we integrated this array and imaging module together with a standard fluidics-handling platform that performs sequential cuvette processing of paramagnetic bead-based ELISA reagents. A standard bead-based immunoassay is performed, and then the beads are transferred to the Simoa imager for signal development and digital quantification.

Fig 2. Low-cost disc micro-arrays make multiplexing feasible.
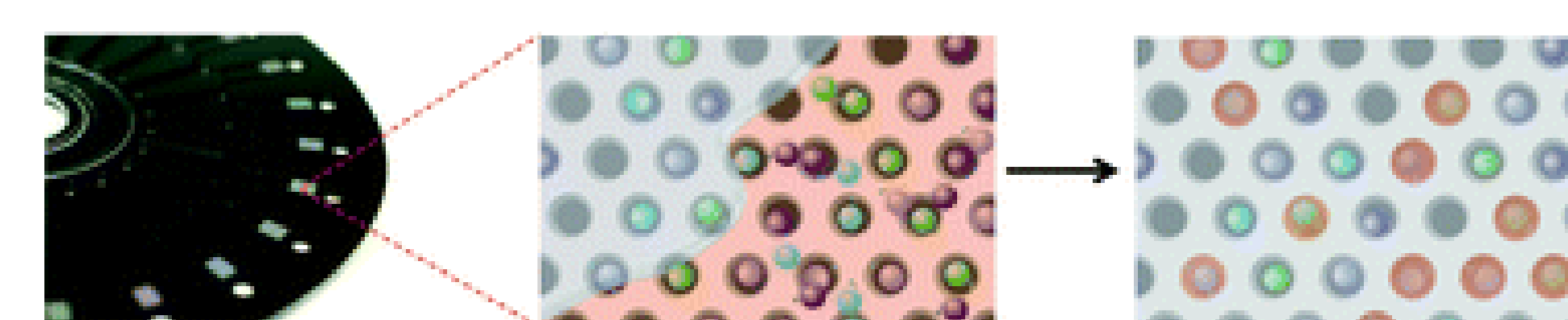
RESULTS

Commercially available single-plex digital immunoassays were developed for a number of markers. LoDs ranged from 0.002 to 0.76 pg/mL (see Table 1). The LoQ of the PSA assay was estimated as 0.037 pg/mL. These sensitivities ranged to over 1000-fold greater than conventional immunoassay. Imprecision for these assays was evaluated over 10 runs across five days in a CLSI format. CVs were generally less than 10%. Spike recovery and linearity met standard criteria for acceptability. The system throughput is 68 tests/hour, and over 4 logs of dynamic range were demonstrated. A prototype cytokine 4-plex was also developed. The 4-plex gave equivalent precision and sensitivity performance to single-plex versions of the same assay.

Fig. 3. Plot of AEB against protein concentration for 4 beads specific to 4 cytokines measured in bovine serum samples spiked with all 4 cytokines.
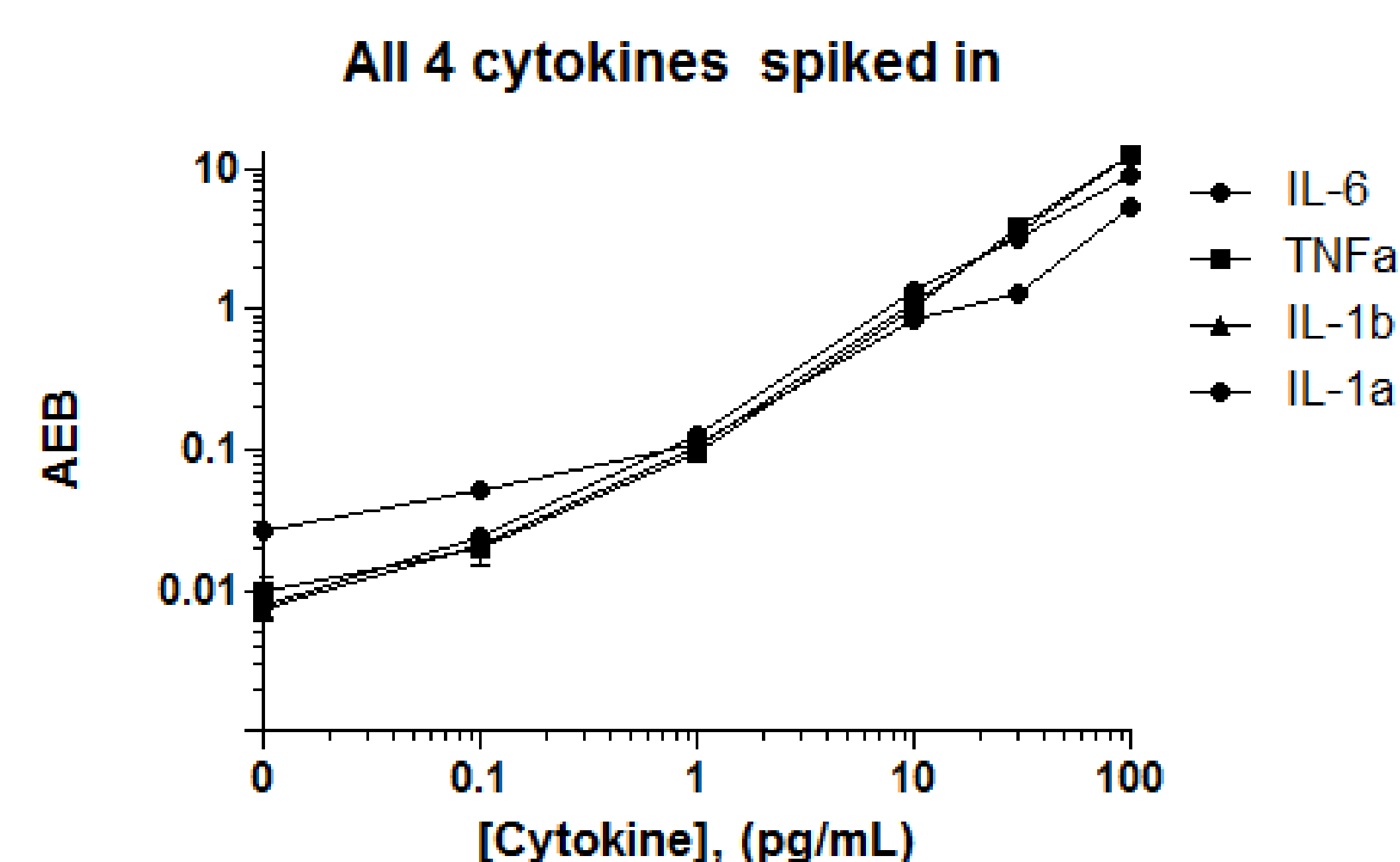

Fig. 4. LoD comparison[1]. Physiological range in samples from normal patients (except where noted) as it compares to the current state-of-the-art technology. The Simoa HD-1 Analyzer demonstrates considerable improvement in the LoDs of these representative assays.

Legend:
- Current state-of-the-art technology (2.5 SD)
- Quanterix LoD (2.5 SD)
- Lower normal plasma or clinical* ranges (median)
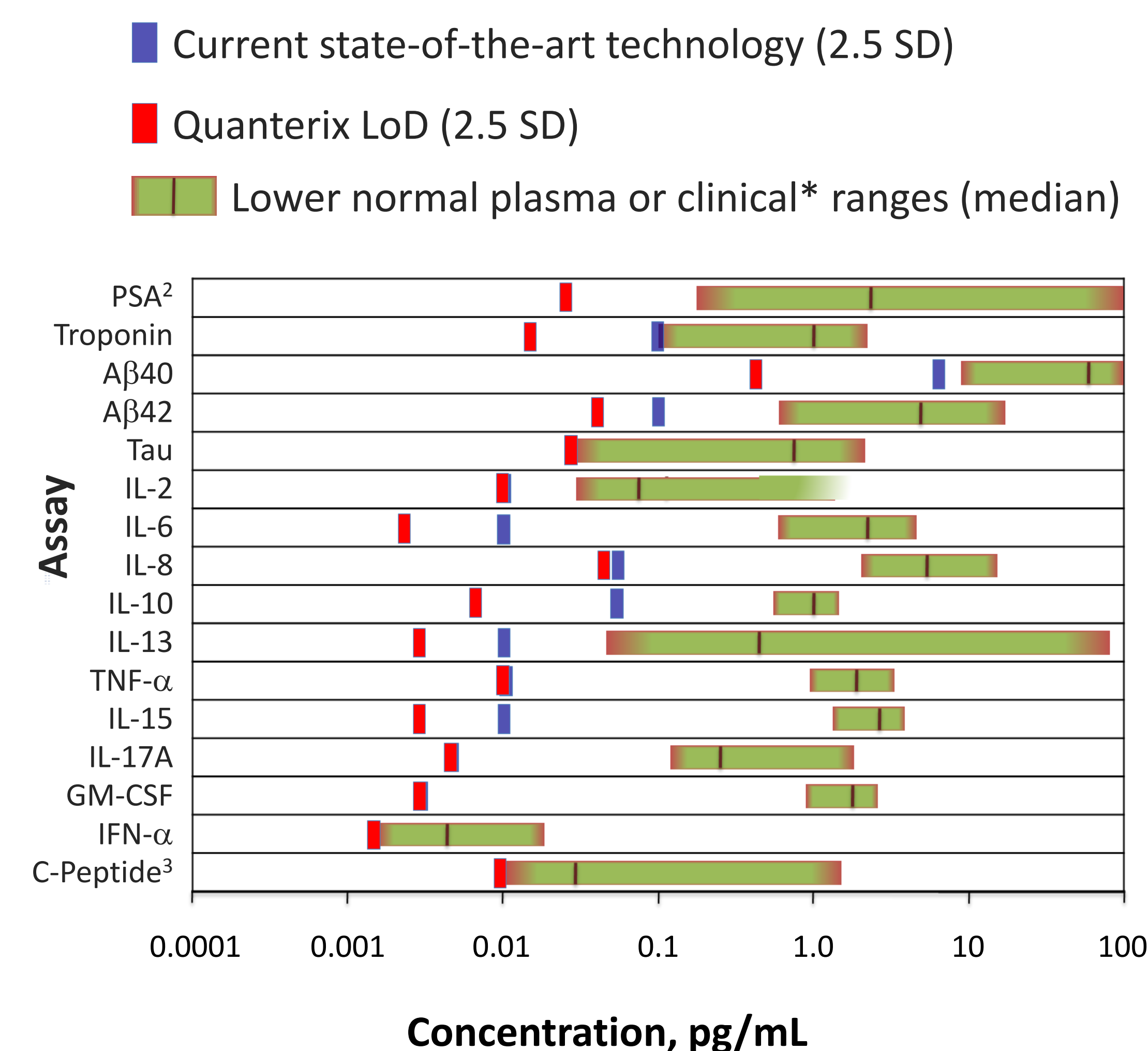
[1] Data from manufacturers' product data sheets, 26Apr2014
[2] Range depicted: post radical prostatectomy patients
[3] Range depicted: juvenile diabetes

Fig. 5. [C-Peptide] in EDTA plasma (n = 12), matched serum (n = 12), and serum from Type I diabetes patients (n = 29). Of 38 Type I samples tested, 5 were unreadable and 4 were >400 pg/mL. Error bars depict mean and SEM.
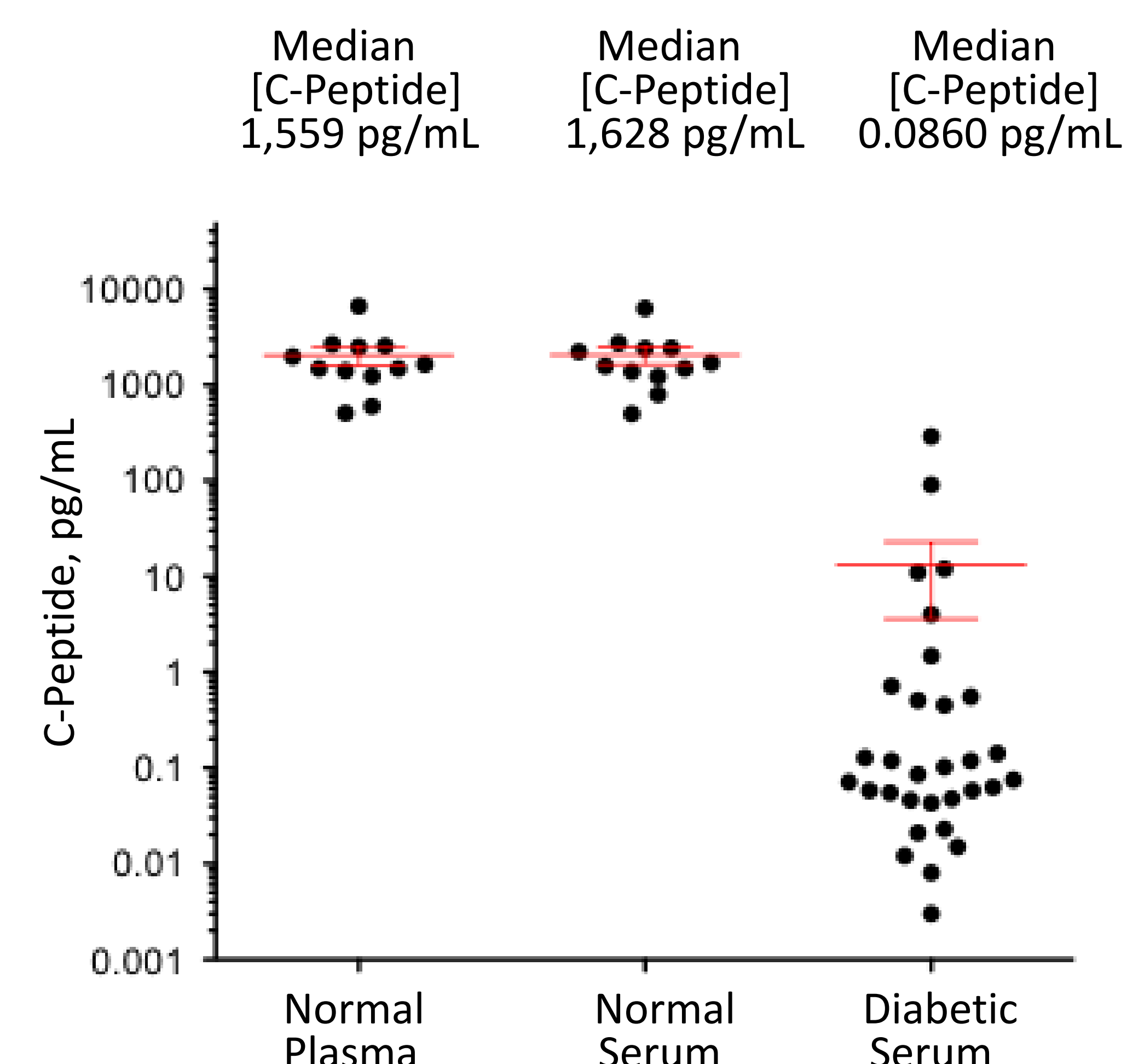
Median [C-Peptide] 1,559 pg/mL	Median [C-Peptide] 1,628 pg/mL	Median [C-Peptide] 0.0860 pg/mL

Table 1. General and precision characteristics of Simoa Human immunoassays

Assay	Dynamic Range (pg/mL)	LoD[1] (pg/mL)	LoQ[2] (pg/mL)	Precision Range (% CV)
Aß40	0–400	0.76	1.18	2.4–8.0
Aß42	0–400	0.034	0.053	3.9–10.0
C-Peptide	0–400	0.013	0.021	3.1–9.1
GM-CSF	0–120	0.0035	0.0079	5.0–14.2
IFNα	0–16.8	0.0016	0.0029	5.0–9.0
IL-2	0–400	0.0105	0.10	8.6–9.3
IL-6	0–120	0.0020	0.0031	5.0–11.9
IL-7	0–400	0.015	0.046	2.9–9.4
IL-8	0–1200	0.0560	0.0921	3.8–7.8
Il-10	0–120	NA	0.0047	3.5–12.7
IL-13	0–300	0.0031	0.0114	4.7–6.2
IL-15	0–40	0.0030	0.0062	4.6–11.9
IL-17A	0–120	NA	0.0041	4.2–6.1
TNFα	0–200	0.014	ND	2.8–5.5
Total PSA	0–400	0.020	0.037	5.0–10.0
Total Tau	0–400	0.019	0.023	3.8–11.3
TRAIL	0–400	0.0083	0.0177	4.5–6.9
Troponin-I	0–1200	0.010	0.079	4.8–9.7

[1] Lower limit of detection (2.5 SD from zero, n=3 reps × 10 runs over 5 days, mean LoD)

[2] Lower limit of quantification ≤20% CV$_{dose}$

CONCLUSIONS

The data indicate we have developed a next generation fully automated immunoassay analyzer capable of orders-of-magnitude greater sensitivity than conventional state-of-the-art immunoassay systems.

REFERENCES

Rissin DM, et al. (2010) Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nature Biotech. 28: 595-99.

Rissin DM, et al. (2011) Simultaneous detection of single molecules and singulated ensembles of molecules enables immunoassays with broad dynamic range. Anal Chem. 83: 2279-85.

Chang L , et al. (2012) Single molecule enzyme-linked immunosorbent assays: Theoretical considerations. J Immun Meth. 378: P112-15.

Rissin DM, et al. (2013) Multiplexed single molecule immunoassays. Lab on a Chip. 13: 2902-2911.

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