Comparison of two platforms quantitating fg/mL biomarkers using single molecule arrays and digital ELISA: the benchtop reader SR-X™, and the fully automated analyzer HD-1™

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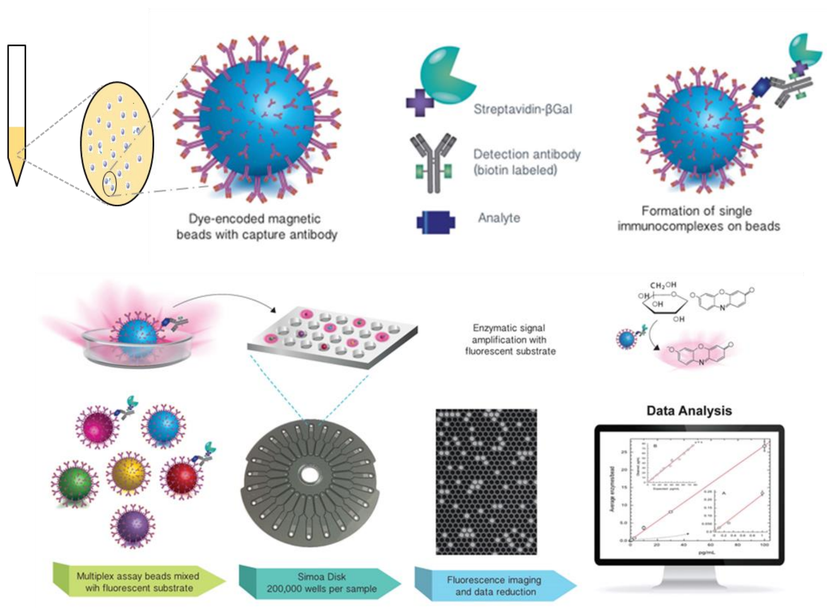
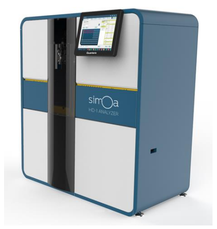
Abstract

Digital ELISA (Enzyme Linked Immunosorbent Assay) based on single molecule arrays (Simoa) has improved sensitivity of traditional ELISA from picomolar (10^{-12} M) to femtomolar (10^{-15} M), increasing the quality and quantity of biomarkers that can be measured for health and disease. Digital ELISA counts signal generated from single immunocomplexes formed on superparamagnetic beads confined in arrays of femtoliter-sized wells in which fluorescent molecules are highly concentrated. We have commercialized digital ELISA in a fully-automated instrument (Simoa HD- Analyzer), ideal for use in pharmaceutical companies, drug discovery, clinical research and other areas necessitating full automation and high throughput. We have recently launched the SR-X benchtop reader, with a smaller footprint and more flexible workflow. Operators prepare assays in microtiter plates at the bench in a semi-automated format similar to traditional ELISA, with the notable exception that plates are preserved by drying after assay completion, and can be read immediately or the next day.

Study Objective: Cross Validation of SR-X and HD-1. We have compared performance of the following Simoa assays on SR-X to HD-: Aβ40; Aβ42; Cytokine 6-Plex Panel ; HIV p24; IFNγ; IL-6; IL-10; IL-13; IL-17A; IL-22; IL-33; mouse Tau; Neurology 4-Plex A; Neurofilament-light; PD-L1; PSA; Tau; and TNFα. Measured sample levels correlated with R2 values from 0.96 to .00, with average LOD and LLOQ within .4 and .5 fold of HD-, respectively. Inter-assay precision ranged from 4.0 to .2% CV across assays. Operators tested full-plates from start to finish within – 2 hours (/2 hour hands on time) and a read time of 2 hours (5 minutes hands on time). The more flexible work-flow of SR-X allows exploration of novel uses of Simoa, including nucleic acid testing.





HD-1

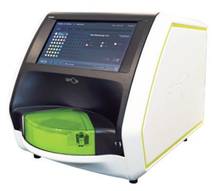
Floor standing

Fully automated sample processing

- Scan barcoded reagents and place directly on instrument
- Samples are processed serially through full ELISA assay

Applications

- Higher throughput sample processing with Assay kits or Homebrew assays
- Running assays under regulatory compliance



SR-X

Benchtop Instrument

Offline Assay

- Semi-automated sample processing on plate washer
- Flexible assay parameters
- Beads are dried prior to reading

Applications

- Lower throughput sample processing with Assay kits or Homebrew assays
- Reagent screening prior to HD-1 assay optimization
- Novel assay development

Results

Fig. 1. SR-X workflow. The assay incubation and wash steps are similar to a standard ELISA plate protocol. Reagents are added using a multi-channel pipette. Beads are re-suspended using a microplate shaker and plates are washed using an automated magnetic microplate washer.

Fig. 2. HIV p24 calibration curves illustrate typical Simoa assay range. Average Enzyme per Bead (AEB) is plotted as a function of analyte concentration, from 16 fg/mL up to 30 pg/mL.

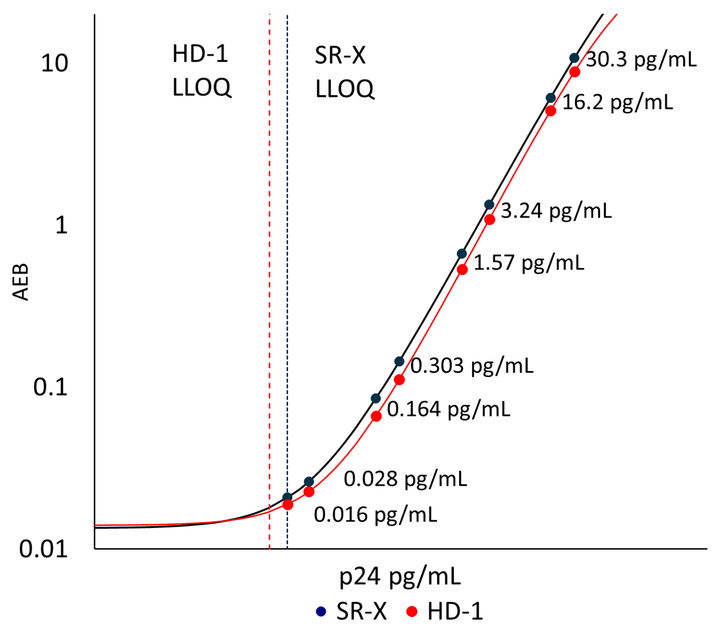


Fig. 3 Cross Platform Sample Correlation. Ten matched serum and plasma samples from normal donors were measured on SR-X and HD-1 across 26 markers. Sample levels over 10 orders of magnitude correlated with an average R^2 of 0.9412.

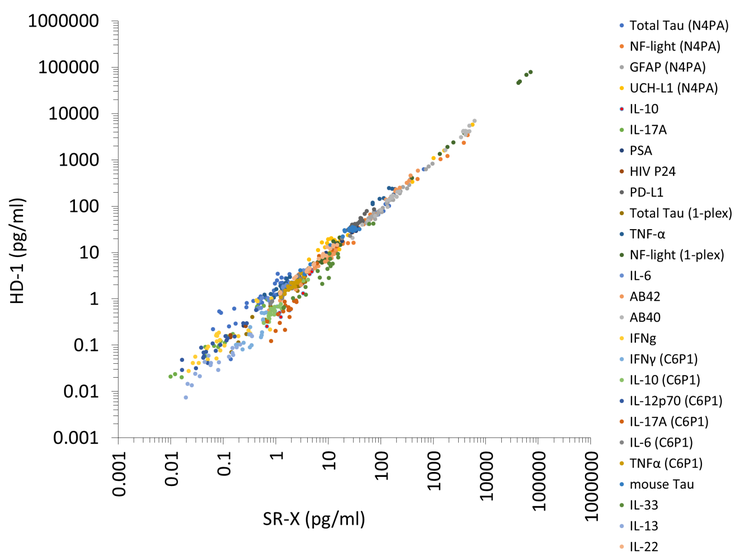
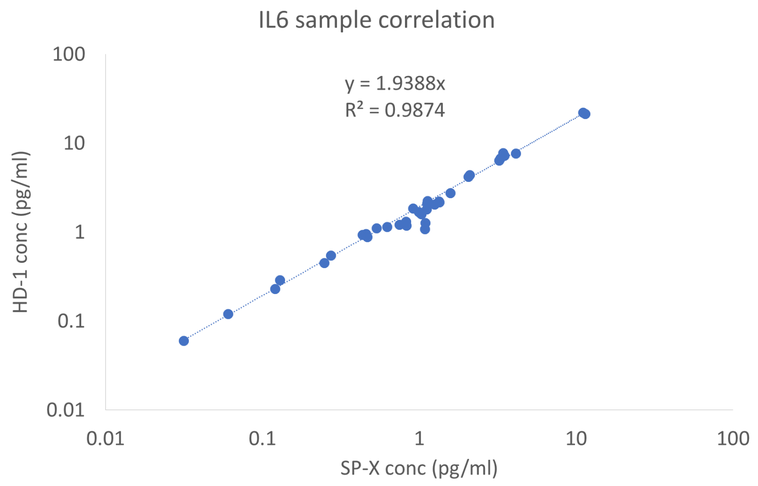


Table 1. Performance characteristics of SR-X and HD-1. Limit of detection (LOD), lower limit of quantitation (LLOQ), intra-run and inter-run precision were compared for SR-X and HD-1. LOD was estimated as 2.5 standard deviations above the blank. LLOQ was determined as the lowest dilution of calibrator that showed CV < 20% and accuracy within 20%. Intra-run and Inter-run precision were measured with two controls and three serum / plasma panels over 6 runs across 2 instruments. SR-X LOD was 98% and LLOQ was 134% compared to HD-1 on average. Average intra-run precision was 6.4% and 6.0% for SR-X and HD-1 respectively; average inter-run precision was 7.0% and 6.0% for SR-X and HD-1 respectively.

	LOD (pg/mL)		LOQ (pg/mL)		Intra-Run Precision		Inter-Run Precision	
Biomarker	SR-X	HD-1	SR-X	HD-1	SR-X	HD-1	SR-X	HD-1
Aβ40	0.170	0.522	4.94	4.92	3.7%	6.8%	5.3%	4.0%
Aβ42	0.035	0.044	0.55	0.55	5.2%	6.6%	10.9%	7.7%
IFNγ (C6P1)	0.016	0.020	0.51	0.26	8.7%	6.2%	7.6%	5.7%
IL-10 (C6P1)	0.009	0.023	0.18	0.09	5.3%	5.9%	6.1%	7.3%
IL-12p70 (C6P1)	0.008	0.021	0.17	0.08	6.5%	5.4%	5.1%	4.4%
IL-17A (C6P1)	0.041	0.099	0.79	0.40	7.9%	5.1%	6.9%	4.1%
IL-6 (C6P1)	0.012	0.085	0.34	0.34	5.0%	4.9%	4.9%	5.9%
TNFα (C6P1)	0.012	0.031	0.12	0.12	7.6%	5.8%	6.3%	5.0%
HIV P24	0.005	0.003	0.02	0.01	8.6%	5.9%	6.8%	4.0%
IFNγ	0.014	0.012	0.20	1.22	9.4%	7.2%	9.6%	3.9%
IL-6	0.006	0.006	0.04	0.04	4.9%	7.1%	2.3%	2.2%
IL-10	0.005	0.004	0.16	0.08	5.4%	4.6%	3.9%	6.2%
IL-13	0.001	0.002	0.01	0.01	7.5%	6.6%	7.1%	7.7%
IL-17A	0.011	0.004	0.04	0.08	5.9%	4.7%	6.8%	5.0%
IL-22	0.005	0.005	0.08	0.04	3.5%	6.4%	4.1%	6.6%
IL-33	0.343	0.320	2.74	1.37	6.8%	5.5%	8.5%	5.7%
mouse Tau	0.428	0.615	3.29	13.17	7.4%	7.1%	10.3%	11.7%
NF-light (N4PA)	0.136	0.104	1.27	0.96	4.5%	5.1%	4.4%	5.2%
Tau (N4PA)	0.030	0.024	0.46	0.21	4.0%	5.8%	3.8%	4.3%
GFAP (N4PA)	0.276	0.221	3.73	1.87	8.2%	4.8%	6.0%	4.8%
UCH-L1 (N4PA)	4.029	1.740	38.40	21.80	11.2%	7.2%	9.0%	5.0%
NF-light	0.073	0.040	1.37	0.68	6.5%	7.8%	9.0%	10.2%
PD-L1	0.048	0.055	1.04	12.30	4.1%	5.5%	9.0%	7.6%
PSA	0.006	0.015	0.11	0.10	5.3%	6.6%	8.1%	7.4%
Total Tau	0.015	0.019	0.20	0.24	9.0%	7.1%	10.1%	8.6%
TNF-α	0.004	0.016	0.07	0.14	5.4%	4.0%	9.7%	5.5%

Correlation with SP-X Array

Fig. 4. Quanterix has recently commercialized a new, micro-spot immunoassay instrument (SP-X), based on antibody arrays. Features include multiplexing up to 10 analytes, with fg/mL sensitivity. Sample readings correlate between the Simoa bead-based platforms and the micro-spot array system with an R^2 of 0.9874 for IL-6, shown here.



Conclusions

The Quanterix SR-X benchtop single molecule detection system provides performance equivalent to the fully-automated HD-1 Analyzer. The more compact benchtop form factor reduces the physical space and the semi-automated workflow of the SR-X has been optimized using conventional laboratory sample prep devices. The bead-based Simoa assays are well correlated with micro-spot based assays providing researches with a new method for ultra-sensitive biomarker detection.

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