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.

Methods

Cross Validation of SR-X and HD-L. We have compared performance of the following Simoa assays on SR-X to HD: IL-1β, TNF-α, Cytokine 6-plex Panel; HIV p24, PTN, IL-6, IL-10, IL-13, IL-17, IL-17A, IL-17F, IL-22, IL-3; mouse Tau, Neurotrophin 4-plex A; Neurotrophin-light; PD-L1; PSA, Tau, and TMA. Measured sample levels correlated with R^2 values from 0.95 to 0.99, with average LOD and LLOQ within 4 and 3 fold of HD, respectively. Inter-assay precision ranged from 4.0% to 5.5% CV across assays. Operators tested full-plates from start to finish within ~2 hours (on hands on time) and a real time of 2 hours (minutes hands on time). The more flexible workflow of SR-X allows exploration of novel uses of Simoa including nuclear acid testing.

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.

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. LOD was determined as the lowest dilution of calibrator that showed CV < 10% and accuracy within 10%. 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 114% compared to HD-1 on average. Average intra-run precision was 6.4% and SR-X LOD and HD-1 respectively, average inter-run precision was 7.0% and 6.0% for SR-X and HD-1 respectively.

Results

We have optimized an anti-Tau sensor on SR-X and compared to HD-1. The Tau sensor has been developed to measure levels of Tau in neurologic disorders such as Alzheimer's disease. This sensor has been optimized to demonstrate the feasibility and utility of SR-X in a high throughput, automated format.

Fig. 2. Cross Platform Sample Correlation. The measured 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 0.941.

Summary

The SR-X benchtop single molecule detection system provides performance equivalent to the fully-automated HD-1 Analyzer for ultra-sensitive detection of fluid biomarkers at high concentrations in single-plex and multi-plex assay formats. The more compact benchtop format further reduces the physical space and can be used in an automated workflow of the SR-X has been optimized using conventional laboratory sample prep devices.