Development of an Ultra-Sensitive Digital Immunoassay (Simoa) to Detect PSD-95 in Cerebrospinal Fluid (CSF) and Brain Lysate

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BACKGROUND

PSD-95 (postsynaptic density protein 95) is a membrane-associated guanylate kinase (MAGUK) scaffolding protein encoded by the DLG4 gene and is associated with excitatory synapses. This protein is an emerging biomarker in Alzheimer's disease (AD) research as recent studies have correlated a decline of PSD-95 with neuronal loss, behavioral decline, and other measures of synaptic plasticity. [3, 4, 5]

METHODOLOGY

We developed an ultrasensitive digital immunoassay using single molecule array (Simoa) technology for the purpose of measuring PSD-95 in both human cerebrospinal fluid (CSF) and mouse brain lysates. Anti-PSD-95 capture beads were prepared by covalent coupling of antibody (mouse anti-PSD-95 mAb) to carboxyl paramagnetic microbeads and detector antibody (rabbit anti-PSD-95 mAb) was biotinylated by standard methods. The HD-1 Analyzer first performs a 2-step sandwich immunoassay with 100 µL of CSF or brain lysate (first step combines capture beads, sample, and biotinylated antibody to form sandwich complex; after incubation and wash, streptavidin beta–galactosidase (SβG) is added to cuvettes to label the complex). After fully-labeled complex is formed, beads are washed and transferred to a Simoa disc where the beads are isolated in femtoliter-sized microwells, sealed in the presence of substrate (resorufin β-d-galactopyranoside, RGP), and analyzed for presence of enzyme label. A single labeled PSD-95 molecule provides sufficient fluorescence signal in 30 seconds to be counted by the HD-1 optical system. [6, 7, 8]

At low PSD-95 concentrations the percentage bead-containing wells in the array with a positive signal is proportional to the amount of PSD-95 present in the sample; at higher concentrations, the total fluorescence signal is proportional to the PSD-95 in the sample. Raw data are output as "average enzymes per bead" (AEB values). The concentration of PSD-95 in samples is then interpolated from a standard curve (4-parameter curve fit, $1/y^2$ weighting).

The Limit of detection (LOD) was determined from calibration curves prepared using recombinant protein. Mouse brain lysate (PS19 human tau-transgenic) and human CSF samples were used to evaluate dilutional linearity, parallelism, spike recovery, and assay sensitivity. Using this digital ELISA, PSD-95 levels were evaluated in human CSF samples from both AD patients and healthy volunteers (HV).

RESULTS

Fig. 1. Representative dose response of Simoa PSD-95 assay across a 4-log dynamic range. Each data point represents the mean of 2 replicates. The limit of detection (2.5 SD above background) is 0.30 pg/mL.

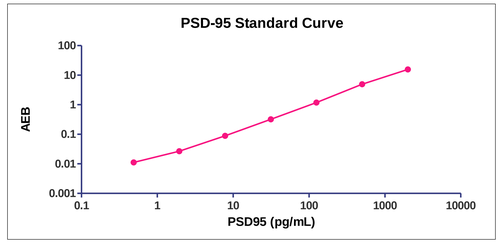


Fig. 2a. Linearity of Simoa PSD-95 assay. Linearity was conducted by serial dilution of pooled CSF samples (Healthy Volunteer [HV] or Alzheimer's Disease [AD]) spiked with antigen. Baseline dilution was 8-fold. Mean dilution linearity of spiked CSF was 94% compared to 8-fold baseline dilution. Dotted lines in both figures represent optimal recovery range (100 ± 20%).

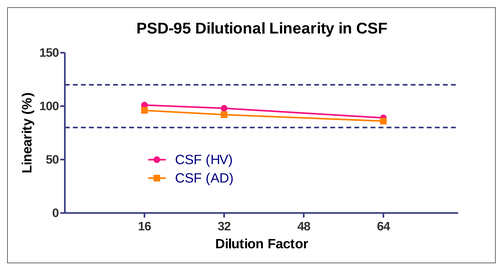


Fig. 2b. Spike recovery of Simoa PSD-95 assay. Spike recovery is calculated by spiking multiple levels of antigen into normal CSF samples (diluted 4-fold). Recovery of PSD-95 spike averaged 105%.

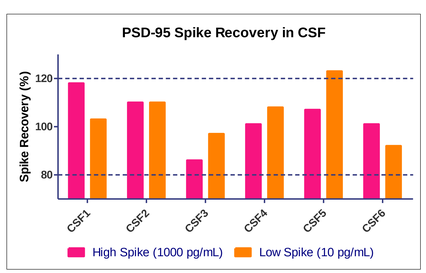


Fig. 3. Parallelism of human CSF in Simoa PSD-95 assay. Parallelism was conducted by serial dilution of human CSF samples (10 HV, 10 AD samples) and compared to baseline dilution of 8-fold. Mean parallelism in samples was 97%.

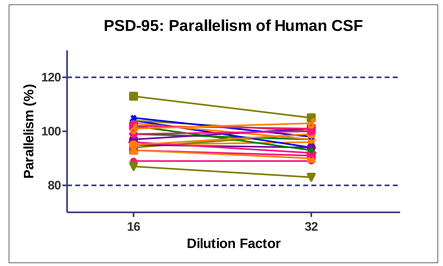


Fig. 4. Parallelism of mouse brain lysate in Simoa PSD-95 assay. Parallelism was conducted by serial dilution of PS19 human tau-transgenic mouse brain lysate. Mean endogenous PSD-95 parallelism was 109%. Baseline dilution was 100-fold.

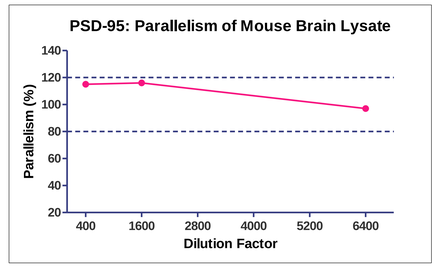
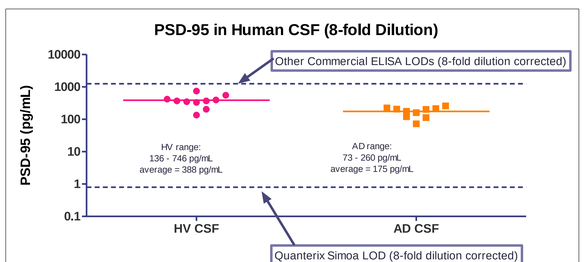


Fig. 5. Concentrations of endogenous PSD-95 in human CSF (AD and HV) samples using Simoa PSD-95. All CSF samples have been age-matched from elderly populations. All CSF samples tested were quantifiable, with AD samples ranging from 72 – 295 pg/mL (mean 175 pg/mL) and HV samples ranging from 121 – 746 pg/mL (mean 388 pg/mL) (after dilution correction). Using an unpaired t test, there is a statistical difference between the two groups (p-value = 0.0015).



Other commercial ELISA kits for detecting PSD-95 in human/mouse samples have LODs ranging between 156 – 313 pg/mL, uncorrected for dilution factor. The figure shows CSF samples tested with Simoa; the upper dotted line represents the lowest LOD (8-fold corrected) from other commercial kits, whereas the lower dotted line is the 8-fold corrected LOD on Simoa

CONCLUSIONS

The ultra-sensitive Simoa assay may be used to detect PSD-95 in CSF and brain lysates to help facilitate research in AD and potentially other neurological diseases. Simoa technology was able to detect endogenous PSD-95 in all human CSF samples tested, which may have tested below the LODs of other commercial ELISAs. Simoa was also able to detect PSD-95 reliably and specifically (shown in linearity and spike recovery testing) and show a statistical difference between HV and AD samples.

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