Development of an Ultrasensitive Digital Immunoassay for the Measurement of LRRK2 in Cerebral Spinal Fluid (CSF)

Nathan Estochen[1], B.S., Dipika Gemani[1], M.S., Melissa Berman[2], M.S., Linan Song[1], Ph.D, David Hanlon[1], Ph.D, Andreas Jeromin[1], Ph.D, Danielle Graham[2], Ph.D.
[1]Quanterix Corporation, Lexington, MA, USA
[2]Biogen, Cambridge, MA, USA

Quanterix™
The Science of Precision Health

BACKGROUND

Parkinson's disease (PD), like other neurological disorders, has been recognized to have a genetic component. Mutations in the leucine-rich repeat kinase-2 (LRRK2) gene are recognized as genetic risk factors for familial PD, and may also represent causal factors in the more common sporadic form of the disease. The central role of LRRK2 in genetic and idiopathic PD has led to significant interest in further characterizing LRRK2 at both the genetic and protein levels.

METHODOLOGY

We developed an ultrasensitive digital immunoassay utilizing single molecule array (Simoa) technology for the purpose of measuring LRRK2 in human cerebrospinal fluid (CSF), peripheral blood mononuclear cells (PBMC) as well as rat and human brain lysate. Anti-LRRK2 capture beads were prepared by covalent coupling of antibody (Biolegend, Cat # 808201) to carboxyl paramagnetic microbeads, detector antibody (Neuromab Cat # 75-253) was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and beta–galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 100 µL of CSF or lysate sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled LRRK2 molecule provides sufficient fluorescence signal in 30 seconds to be counted by the HD-1 optical system. At low LRRK2 concentration, the percentage bead-containing wells in the array with a positive signal is proportional to the amount of LRRK2 present in the sample. At higher LRRK2 concentration, the total fluorescence signal is proportional to the LRRK2 in the sample. The concentration of LRRK2 is then interpolated from a standard curve. Limit of detection (LOD) was determined from recombinant protein calibration curves. Human CSF and brain lysates were used to evaluate dilutional linearity and assay sensitivity. Spike recovery of recombinant LRRK2 protein was evaluated for measurement accuracy in sample matrix. Cross reactivity was also tested between LRRK2 and LRRK1 recombinant proteins. Using this digital ELISA, LRRK2 levels in PBMC (data not shown) and brain lysates were determined and compared with the results from the Singulex Erenna (Capture: Biolegend Cat # 844401, Detector: Abcam Cat # ab195023) platform.

RESULTS

Fig. 1. Representative dose response of Simoa LRRK2 assay across a 4 log range. Each data point represents the mean of 3 replicates. The insert highlights the low end of the curve obtained with digital quantification. Limit of detection (2.5 SD) is 1.2 pg/mL.

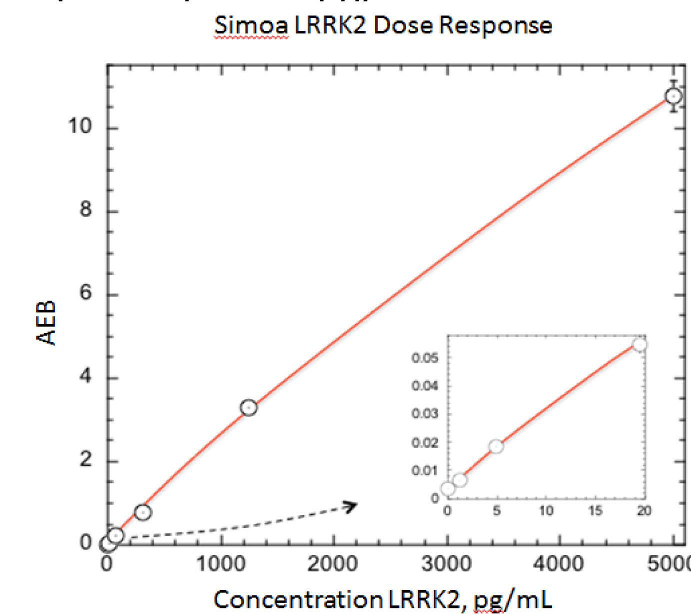
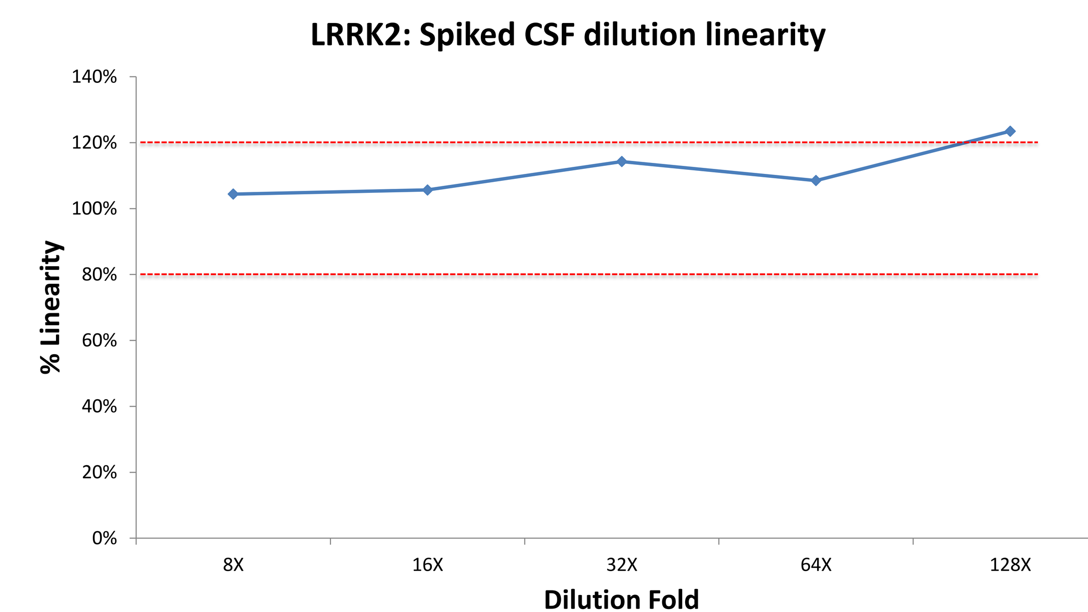


Fig. 2. Linearity and spike recovery of Simoa LRRK2 assay. Linearity was conducted by serial dilution of an individual CSF sample. The mean spike recovery is calculated by spiking multiple levels of antigen into normal CSF samples. Recovery of LRRK2 spike averaged 98.4%. Mean dilution linearity of spiked CSF was 111%.



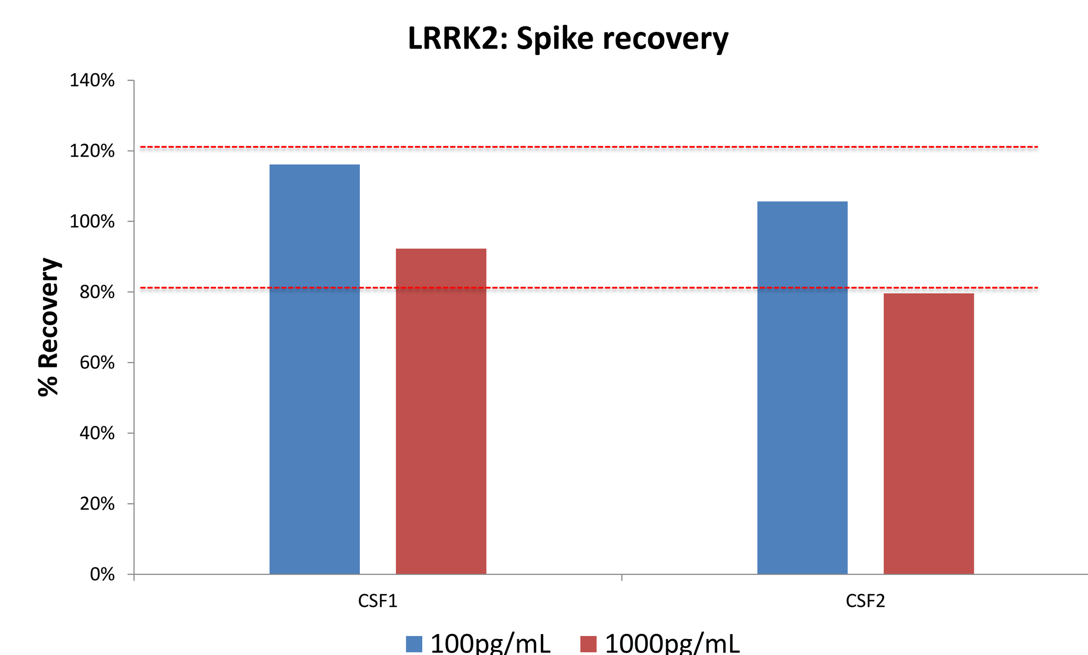


Fig. 3. Parallelism of rat brain lysates of Simoa LRRK2 assay. Parallelism was conducted by serial dilution of brain homogenate of five brain regions of an individual rat. Mean endogenous LRRK2 parallelism of rat brain lysates was 98%.

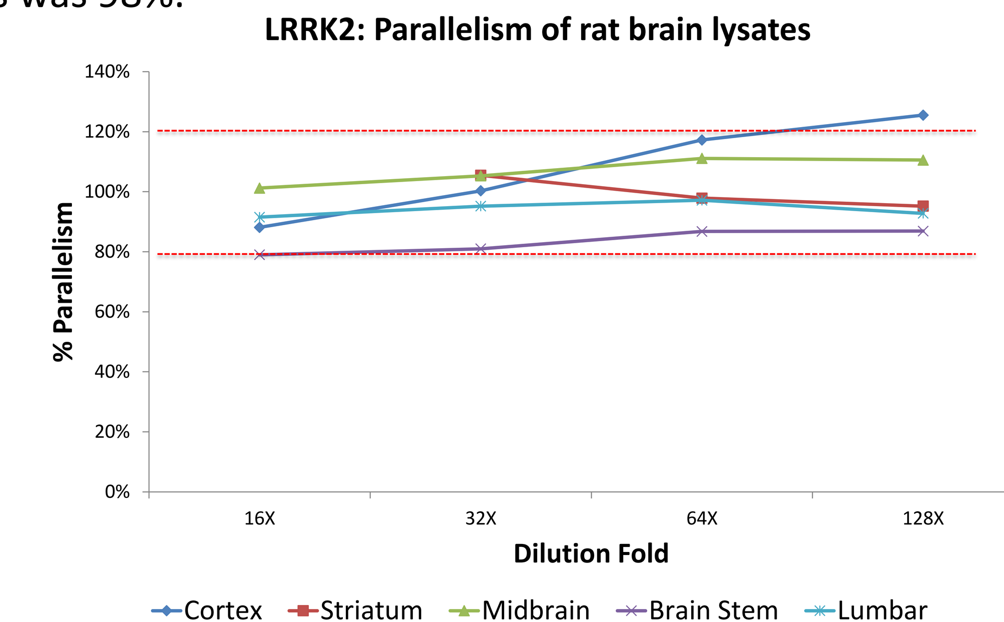


Fig. 4. Parallelism of cynomolgous brain lysates of Simoa LRRK2 assay. Parallelism was conducted by serial dilution of brain homogenate of one brain region for four individuals. Mean endogenous LRRK2 parallelism of cyno brain lysates was 105%.

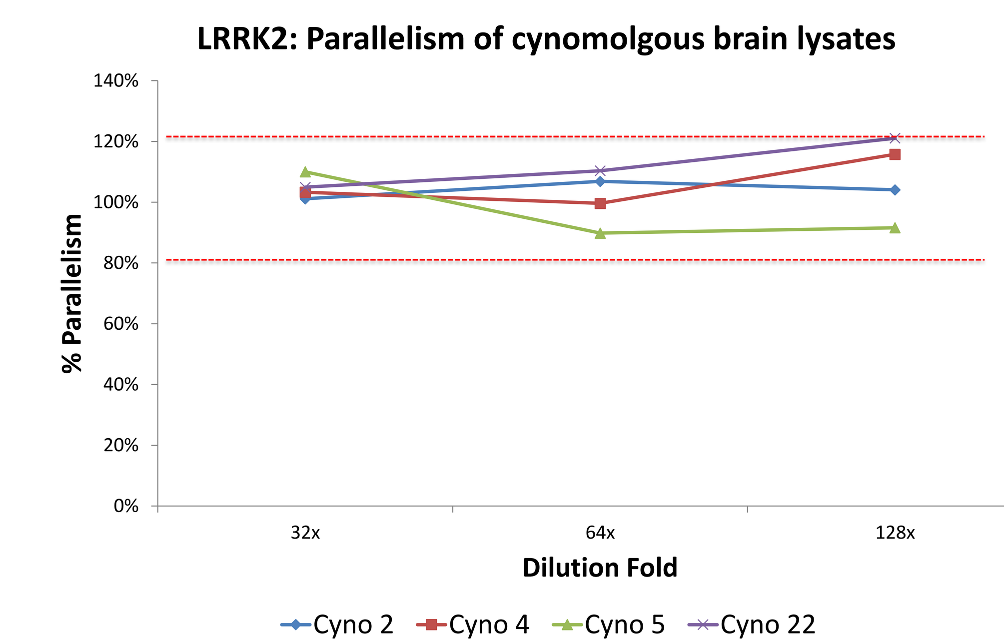


Fig. 5. Correlation of Simoa LRRK2 assay to Singulex Erenna. Wildtype, transgenic and knockout mice brain lysates were tested and correlated between Simoa and Erenna platforms.

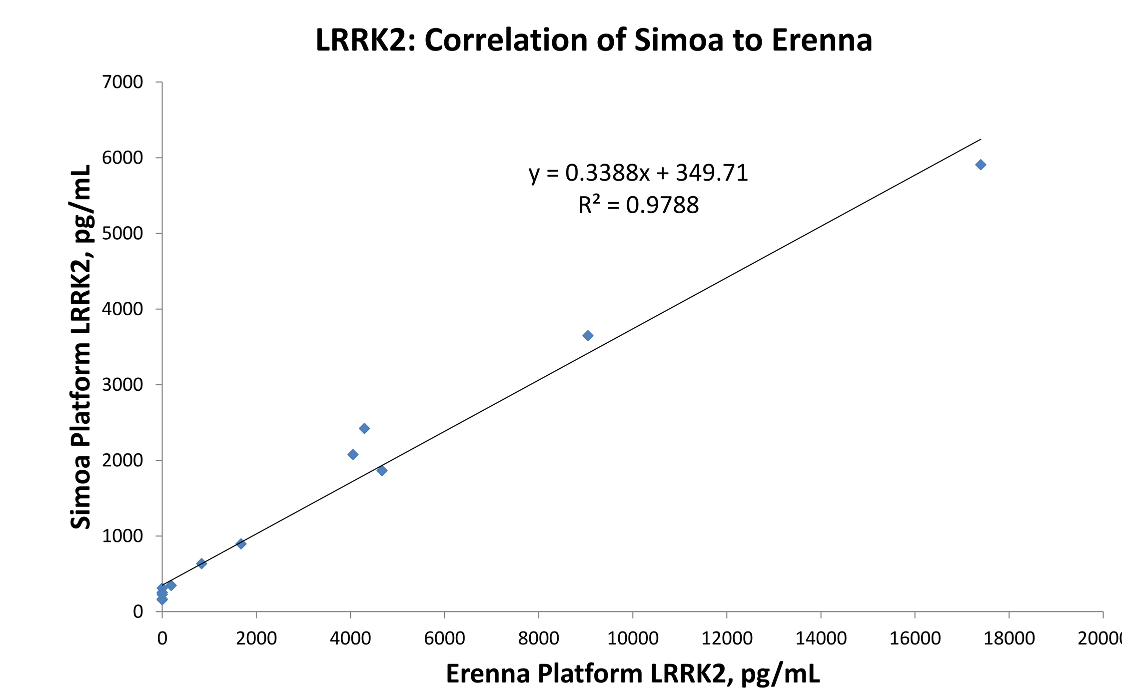
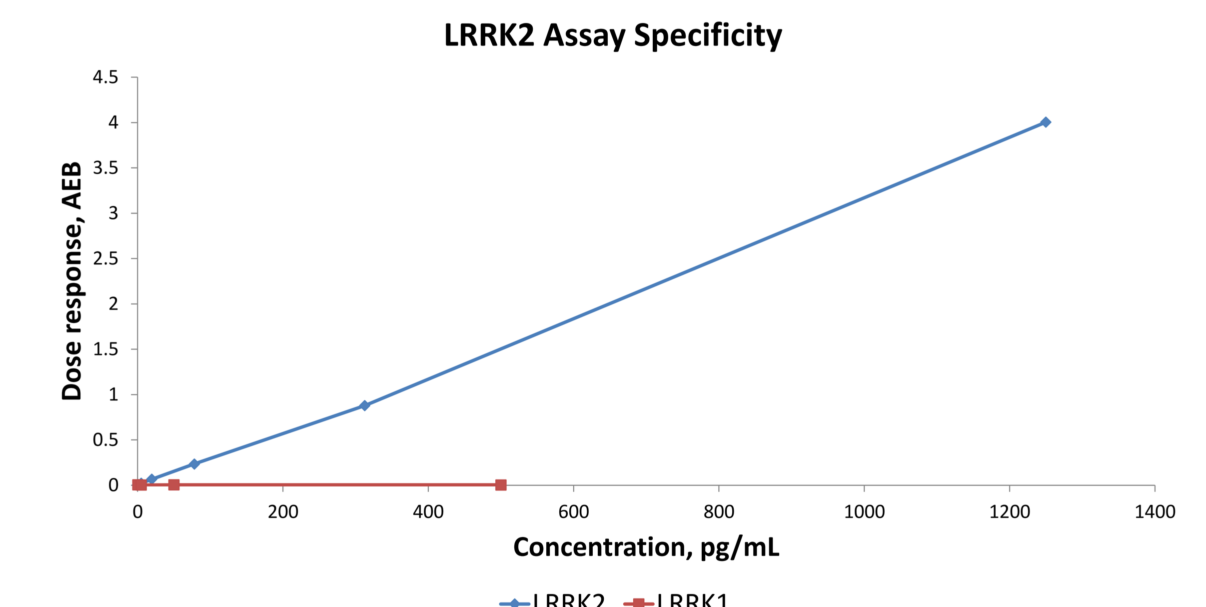


Fig. 6. Cross reactivity of LRRK2 and LRRK1 recombinant proteins. Cross reactivity conducted by assessing AEB signal level response of LRRK2 and LRRK1 calibration curves made from recombinant protein.



CONCLUSIONS

The ultrasensitive digital Simoa assay has potential to accurately quantify LRRK2 in PBMC and brain lysates. Data needs to be replicated with a larger data set including samples from patients diagnosed with PD. Additional assay optimization needs to be performed to reliably detect LRRK2 in CSF.

REFERENCES

Rissin DM, et al. (2010) Single-molecule enzyme-linked immunosorbent assay detects serum proteins at sub-femtomolar 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.

Corresponding author: Nathan Estochen
Quanterix Corporation
113 Hartwell Avenue, Lexington, MA 02421, USA
Tel: 1-617-301-9474
nestochen@quanterix.com
www.quanterix.com