Development of Digital ELISAs for the Ultrasensitive Measurement of Serum Glial Fibrillary Acid Protein and Ubiquitin C-terminal Hydrolase L1 with Clinical Utility in Human Traumatic Brian Injury

Linan Song[1], Ph.D; David Hanlon[1], Ph.D; Dipika Gemani[1], MS; Adam Shepro[1], BS; Andreas Jeromin[1], Ph.D, Aarti Chawla[2], MS, and Michael Catania[2], Ph.D
[1]Quanterix Corporation, Lexington, MA, USA ; [2]Banyan Biomarkers, San Diego, CA, USA

Quanterix™
The Science of Precision Health

BACKGROUND

Several investigations have reported that traumatic brain injury (TBI) is a significant risk factor for neurodegenerative diseases[1]. There is a widely recognized need for sensitive, accurate and rapid blood-based biomarker assays to supplement computed tomography (CT) scans and functional assessments commonly used for acute diagnosis of TBI. Here we developed digital ELISA assays using single molecule array (Simoa) technology for ultrasensitive measurement of serum glial fibrillary acid protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1), respectively.

METHODOLOGY

Six monoclonal antibodies provided by Banyan Biomarkers were first screened to select the best antibody pair for each target protein. Each of the antibodies was coated to paramagnetic beads and also biotinylated during the screening process. Capture beads and biotinylated detector were combined with 100 µL of calibrators or diluted samples, resulting the formation of immunocomplexes on beads. After an initial wash step, beads were mixed with a conjugate of streptavidin-β-galactosidase (SβG) during which SβG bound to the biotin, resulting in enzyme labeled target molecules. Following a final wash, the capture beads were resuspended in a resorufin β-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa array disc, where individual capture beads were loaded and sealed into femtoliter-sized wells for isolation and detection of bound molecules[2]. Average enzyme per bead (AEB) was reported as a measurement of Simoa signal. All the procedures described above were performed on the fully automated Simoa HD-1 Analyzer (Quanterix Corporation)[3].

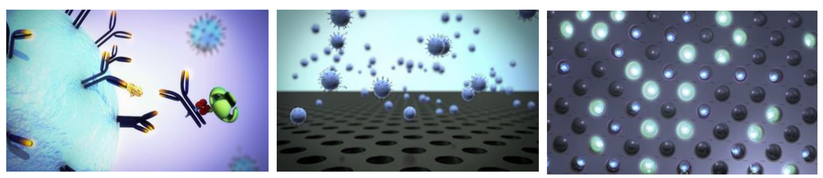


Figure 1. Schematic illustration of Simoa process

RESULTS

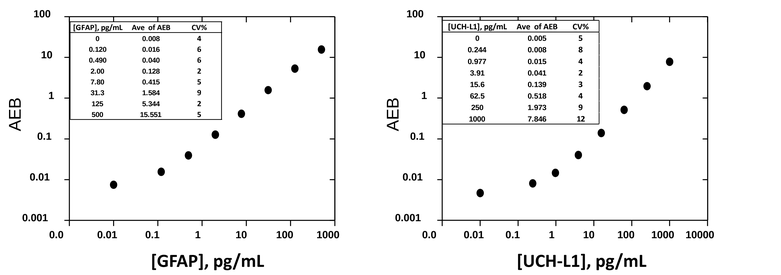


Figure 2. Representative standard curves of digital ELISAs for GFAP (left) and UCH-L1 (right). Each data point represents the average AEB from three replicates.

Table 1. Summary of lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) for both GFAP and UCH-L1 digital ELISAs. LLOD (2.5 SD) and LLOQ (CV and RE <20%) were determined from 6 runs.

	LLOD, pg/mL		LLOQ, pg/mL	
GFAP	0.028	0.112*	0.120	0.480*
UCH-L1	0.157	0.628*	0.244	0.976*

***: accounting for 4x pre-dilutions for samples**

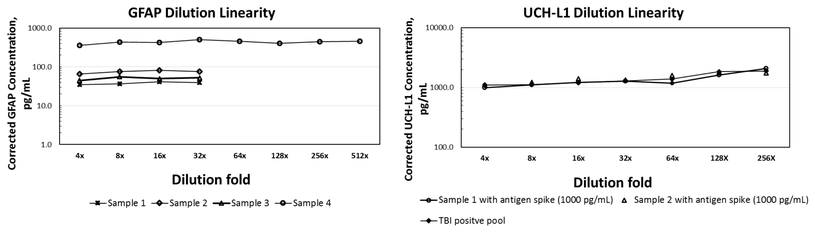


Figure 3. Dilutional linearity of serum GFAP and UCH-L1 using digital ELISA. Dilutional linearity ranged from 106% to 127% (from 4- to 512-fold dilution) with an average of 117% for GFAP and 102% to 143% (4- to 64-fold dilution) with an average of 120% for UCH-L1.

The mean recovery from multiple levels of antigen spiked into normal serum samples was 89.5% for GFAP and 90.5% for UCH-L1.

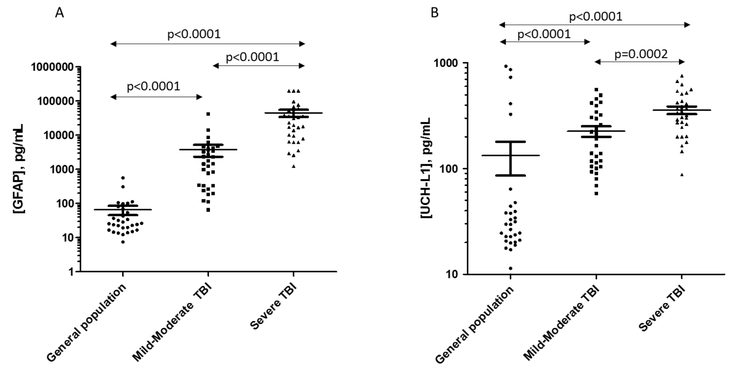


Figure 4. Distribution of serum GFAP (A) and UCH-L1 (B) levels in general population, mild to moderate (GCS of 9-15), and severe (GCS≤8) TBI groups (n=30 for each). All samples were measurable with values above the assay LLOQ for each target. P values were from a Mann Whitney test with 95% CI with a statistical significance cutoff of p<0.05. Analysis of receiver operating characteristic (ROC) curve revealed an AUC (area under the curve) of 0.9733 (0.9401-1.006, 95% CI) for GFAP and 0.8456 (0.7216-0.9695, 95% CI) for UCH-L1 between diagnostic groups of general population and mild-moderate TBI patients.

CONCLUSIONS

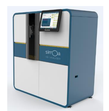
Digital ELISA assays with high sensitivity and accuracy in quantification of both serum GFAP and UCH-L1 provide potential utility in diagnosis of mTBI.

REFERENCES

1. Esopenko C, Levine B. Aging, neurodegenerative disease, and traumatic brain injury: the role of neuroimaging. Journal of neurotrauma. 2015;32(4):209-20.
2. Rissin D, et al. Single-Molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010; 28(6): 595–599.
3. Wilson DH, et al. The Simoa HD-1 Analyzer: a novel fully automated digital immunoassay analyzer with single molecule sensitivity and multiplexing. J Lab Autom. 2015; June 15. doi:10.1177/2211068215589580

Corresponding author: Dr. L Song
lsong@quanterix.com

Quanterix Corporation
113 Hartwell Avenue, Lexington, MA 02421, USA
Tel: 1-617-301-9414
www.quanterix.com



This presented work was supported by the US Army Contracting Command, Aberdeen Proving Ground, Natick Contracting Division, under Contract No. W911QY-15-C-0019