Development of a 4-Plex Digital Immunoassay for Detection of Neurofilament-Light, Tau, Glial Fibrillary Acidic Protein and Ubiquitin c-Terminal Hydrolase L1 in Blood and Cerebrospinal Fluid.

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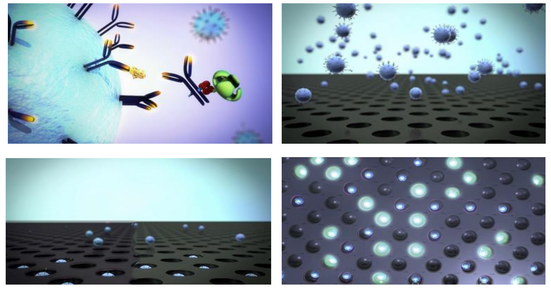
BACKGROUND

Neurofilament Light Chain (NF-Light), Tau, Glia Fibrillary Acidic Protein (GFAP) and Ubiquitin c-Terminal Hydrolase L1 (UCH-L1) are emerging neurology biomarkers relevant for neurodegeneration and traumatic brain injury. Simultaneous measurement of NF-Light, Tau, GFAP and UCH-L1 in serum, plasma, and CSF in both healthy and diseased individuals is of great interest.

METHODOLOGY

A fully automated multiplexed digital immunoassay for these 4 neurology biomarkers was developed based on Single Molecule Array (Simoa) technology with ultra-sensitivity. The assay reagents were designed for use on the Simoa HD-1 Analyzer. In the first step, an immunocomplex was formed by incubating calibrators or samples with capture antibody-coated paramagnetic beads and biotinylated detector antibody. Then, the immunocomplex was labeled with streptavidin-β-galactosidase conjugate followed by incubation with the fluorogenic substrate resorufin-β-D-galactopyranoside. Beads were loaded into a microwell array to produce single beaded wells and digital signals. Multiplexing is achieved by decoding beads with different color of fluorescent labels on beads. Each color of beads is corresponding to a specific antibody therefore a specific target molecule. The individual assays in the plexes were evaluated for sensitivity, linearity, spike/recovery, plex-cross reactivity and detectability in serum, plasma, and CSF from healthy donors. Plasma samples (provided by Dr. Jessica Gill) from military individuals with or w/o blast exposure were tested using the 4-plex assay.



RESULTS

Fig. 1. Representative dose response of Simoa Human Neurology 4-plex 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 0.104 pg/mL (NF-Light), 0.024 pg/mL(Tau), 0.221 pg/mL(GFAP) and 1.74 (UCH-L1) based on 12 individual runs. Four-parameter curve fit parameters are depicted.

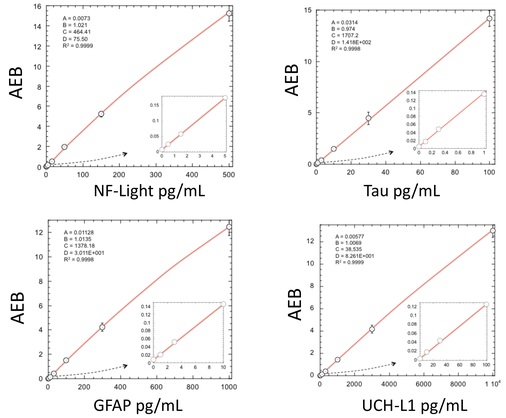


Table 1. Precision characteristics of Simoa Human Neurology 4-Plex assay. Repeatability was determined following guidance of CLSI Protocol EP5-A. 5 samples consisting of 2 serum panels, 1 plasma panel, and 2 controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

Sample	Mean Concentration pg/mL				Between Day %CV, n=5				Between Run %CV n=10				Within Run %CV n=30			
	NF-Light	Tau	GFAP	UCH-L1	NF-Light	Tau	GFAP	UCH-L1	NF-Light	Tau	GFAP	UCH-L1	NF-Light	Tau	GFAP	UCH-L1
Control 1	8.74	3.55	26.2	125	0	0	2.7	0	6.5	6	0	2.8	6.1	6.3	7.2	6.9
Control 2	852	183	1372	11681	2.5	0	5.4	3.6	0	4.6	3.1	3.1	4.3	4.8	4.5	4
Plasma Panel 1	16.1	2.66	60.5	73	0	0	2.6	0	4.4	12.4	2	10.6	6	7.4	5.6	9.6
Serum Panel 2	61.5	20.6	221	356	0	0	0	4	0	7.6	4	6.7	4.6	4.3	4.2	5.6
Plasma Panel 3	325	142	1910	6082	0	0	0	0	7.8	6.2	8.5	7	4.5	4.3	5	4.2

Table 2. Summary of Characterization of Simoa Human Neurology 4-Plex assay.

	NF-Light	Tau	GFAP	UCH-L1
Calibration range	0-500 pg/mL	0-100 pg/mL	0-1000 pg/mL	0-10 ng/mL
Dynamic range	0-2000 pg/mL	0-400 pg/mL	0-4000 pg/mL	0-40 ng/mL
Lower limit of detection (2.5 SD; 3 reps x 12 estimates across 12 runs, 3 instruments, 2 lots; mean LoD)	0.104 pg/mL	0.024 pg/mL	0.221 pg/mL	1.74 pg/mL
Lower limit of quantification (12 runs, 3 instruments, 2 reagent lots, mean LoQ)	0.241 pg/mL	0.053 pg/mL	0.467 pg/mL	5.45 pg/mL
Spike-recovery: serum/plasma (spiked into 5 serum, 5 plasma samples, 2 levels, mean)	90.10%	117.80%	105.00%	95.30%
Spike-recovery: CSF (spiked into 4 CSF samples , 2 levels, mean)	107.70%	116.30%	118.90%	118.90%
Linearity (high plasma sample fractionally admixed with low plasma sample, mean of 10 levels)	94.90%	94.30%	100.60%	95.40%
Dilution linearity: serum (spiked serum diluted 2X serially from MRD to 128-fold w/ Sample Diluent; mean recovery)	118.80%	115.00%	108.30%	90.10%
Dilution linearity: CSF (spiked serum diluted 2X serially from MRD to 128-fold w/ Sample Diluent; mean recovery)	99.90%	91.60%	98.10%	95.80%
Inter lot CV (Pool of CVs from 5 samples tested with 2 reagent lots across 2 runs x 3 instruments)	3.50%	3.00%	4.40%	3.90%
Inter instrument CV (Pool of CVs from 5 samples tested with 3 instruments across 2 runs x 2 reagent lots)	2.50%	3.60%	4.70%	4.50%
Typical serum/plasma sample volume (Includes dead volume; see Package Insert for details)	46 µL	46 µL	46 µL	46 µL
CSF sample volume (Includes dead volume; See Package Insert for details)	4.6 µL	4.6 µL	4.6 µL	4.6 µL

Fig. 2 . Test of Simoa Neurology 4–Plex in normal human samples. [NF-Light], [Tau], [GFAP] and [UCH-L1] in EDTA plasma (n = 20), serum (matched, n = 20) and CSF (non-matched, n = 20) from healthy donors. Error bars depict median and interquartile ranges.

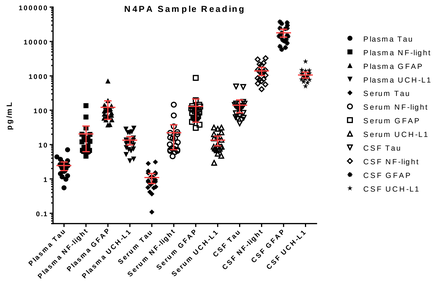


Fig. 3 . Results of Neurology 4-Plex assay showed change of biomarker levels in individuals with and w/o mild Blast exposure. Samples were collected on day 0 (baseline) and day 7 (after exposure). Group 1 includes control individuals with none or less than 2 PSI blast exposure. Group 2 includes individuals with >7 PSI blast exposure. Δ are change of Conc. (pg/mL) between day 0 and day 7 of same individual.

Group		ΔNF-Light	ΔTau	ΔGFAP	ΔUCH-L1
W/O blast exposure (n=24)	Mean	0.34	-0.25	-4.94	-14.95
	SD	10.50	0.58	26.31	18.14
W blast exposure (n=43)	Mean	3.33	0.04	2.60	3.69
	SD	10.07	0.41	19.86	27.80
Total(n=67)	Mean	2.26	-0.06	-0.10	-3.08
	SD	10.25	0.50	22.48	26.19

CONCLUSIONS

The Simoa Human Neurology 4-plex assay is able to reliably measure four biomarkers simultaneously in plasma, serum and CSF samples from healthy individuals. It provides a new tool for the quantification of NF-Light, Tau, GFAP and UCH-L1 in both normal and disease samples, facilitating the investigation of traumatic brain injury and neurodegenerative diseases.

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.

Carr W, et al. (2016) Repeated Low-Level Blast Exposure: A Descriptive Human Subjects Study. Mil Med **181**(5 Suppl): p. 28-39.

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