SimoA Human Neurology 3-Plex A (N3PA) Immunoassay Measures Amyloid beta1-42, Amyloid beta1-40 and Tau in Blood and CSF Samples Simultaneously

Lei Chang, Dandan Shan, Jason Wickman, Marcella Holdridge, Christina Raso, David Wilson
Quanterix Corporation, Lexington, MA

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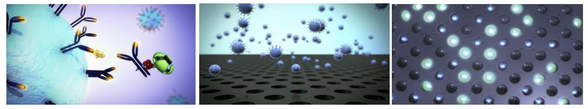
BACKGROUND

Tau and Amyloid beta have been considered as major proteins that related to Alzheimer's disease pathology. Blood Tau only became reliably detected recently. Multiplexed measurement of Amyloid beta1-42 (Aβ 42), Amyloid beta1-40 (Aβ40) and total tau in serum, plasma and CSF continues to be challenging, especially for normal healthy individual. Here we report an automatic multiplexing assay of total Tau, Amyloid beta 1-42 and Amyloid beta 1-40. This fully automated digital immunoassay can measure above 3 biomarkers in serum, plasma and CSF in both healthy and diseased individuals.

METHODOLOGY

A multiplex sandwich immunoassay was developed for Aβ42, Aβ40 and total tau on Simoa HD-1 Analyzer. Simoa Human Neurology 3-plex assay reagents were developed similar to a paramagnetic bead-based ELISA. Anti-tau, anti Amyloid beta 1-42 and anti Amyloid beta 1-40 capture beads were prepared by covalent coupling of target specific antibody to carboxyl paramagnetic microbeads. Detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and beta–galactosidase. The Analyzer performs a 2-step sandwich immunoassay, transfers labeled capture beads to an array of microwells, and interrogates the wells for presence of enzyme label. A single labeled analyte molecule provides sufficient fluorescence in 30 seconds to be detected. The concentration of target is then interpolated from a calibration curve. Multiplexing is achieved by decoding beads with different color of fluorescent labels. Each color is corresponding to a specific antibody therefore a specific target molecule. The multiplex assay was evaluated for sensitivity, precision, linearity, spike/recovery, reproducibility and between-assay-cross activity. Serum, plasma and CSF from healthy donors were evaluated. Alzheimer's Disease and control plasma samples were provided by Dr. Kaj Blennow and tested using Neurology 3-Plex Assay.



RESULTS

Fig. 1. Representative dose response of Simoa Human Neurology 3-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 0.019 pg/mL (Tau), 0.045pg/mL(Aβ42), 0.196 pg/mL(Aβ40) based on 12 individual runs. Four-parameter curve fit parameters are depicted.

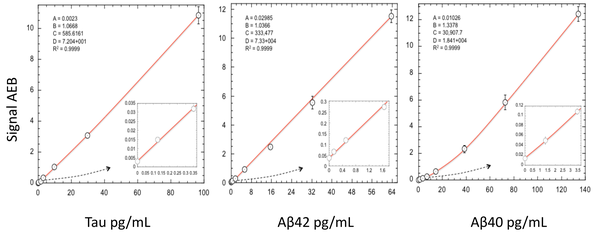


Fig. 2. Dose CV profile. Diluted serum was assayed in reps of 3 over multiple days and run (96 determinations). Concentrations are uncorrected for pre-dilutions.

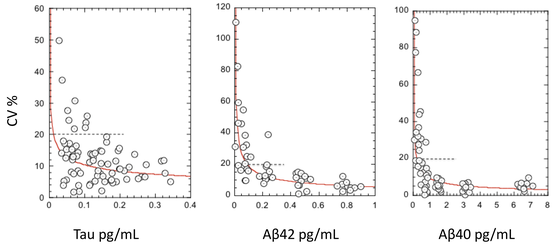


Table 1. Precision characteristics of Simoa Human Neurology 3-Plex assay. Repeatability was determined following guidance of CLSI Protocol EP5-A.

	Mean Concentration pg/mL			Within Run %CV, n=30			Between Run %CV n=10			Between Day %CV n=5		
Sample	**Tau**	**Aβ42**	**Aβ40**	**Tau**	**Aβ42**	**Aβ40**	**Tau**	**Aβ42**	**Aβ40**	**Tau**	**Aβ42**	**Aβ40**
Control 1	2.31	3.12	22.2	9.1	8.1	5.8	0	2.1	1.8	4.4	0	1.7
Control 2	96.6	84.4	378	4.7	5.5	4.1	2.2	2	4.9	4.2	2	1.2
Plasma Panel 1	1.51	3.4	52.9	7.8	6.8	3.5	9.8	0	4	0	5.8	1.7
Serum Panel 2	117	6.06	100	4.8	6.5	4.7	4.2	4.7	7.3	4.2	2.1	0
Plasma Panel 3	2.77	45.3	111	7.7	6.7	5.2	4.9	5.4	3.3	0	2.3	2.1
Serum Panel 4	19.5	147	72.5	3.4	4.3	3.8	4.8	0	0	0	1.8	1.9

Six samples consisting of two serum panels, two plasma panel, and two 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.

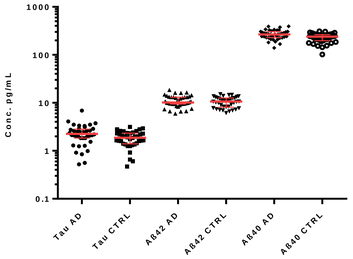
Table 2. Summary of Characterization of Simoa Human Neurology 3-Plex assay.

	Tau	Aβ 42	Aβ 40
Calibration range	0-100 pg/mL	0-60 pg/mL	0-140 pg/mL
Dynamic range	0-400 pg/mL	0-240 pg/mL	0-560 pg/mL
Lower limit of detection (2.5 SD; 3 reps x 12 estimates across 12 runs, 3 instruments, 2 lots; mean LoD)	0.019 pg/mL	0.045 pg/mL	0.196 pg/mL
Lower limit of quantification (12 runs, 3 instruments, 2 reagent lots, mean LoQ)	0.063 pg/mL	0.142 pg/mL	0.675 pg/mL
Spike-recovery: serum/plasma (Spiked into 4 serum, 4 plasma samples at 20 and 200 pg/mL, mean[a])	98.4%	83.0%	111.2%
Spike-recovery: CSF (Spiked into 4 CSF samples at 400 and 4000 pg/mL, mean)	107.4%	116.7%	96.0%
Linearity (high plasma sample fractionally admixed with low plasma sample, mean of 10 levels)	100.3%	93.0%	104.0%
Dilution linearity: serum (spiked serum diluted 2X serially from MRD to 32-fold w/ Sample Diluent; mean recovery)	114.3%	107.0%	112.4%
Dilution linearity: CSF (spiked serum diluted 2X serially from MRD to 128-fold w/ Sample Diluent; mean recovery)	101.4%	98.3%	83.0%
Inter lot CV (Pool of CVs from 6 samples tested with 2 reagent lots across 2 runs x 3 instruments)	3.5%	3.5%	3.5%
Inter instrument CV (Pool of CVs from 6 samples tested with 3 instruments across 2 runs x 2 reagent lots)	4.1%	4.1%	4.1%
Typical serum/plasma sample volume (Includes dead volume)	46 µL	46 µL	46 mL
Minimum serum/plasma sample volume (Depends on dilution procedure)	13.5 µL	13.5 µL	13.5 mL
CSF sample volume (Includes dead volume)	2.3 µL	2.3 µL	2.3 mL

Table 3. Test of normal human samples using Simoa Neurology 3–Plex assay.

Samples	Plasma			Serum			CSF		
	Tau	**Aβ42**	**Aβ40**	**Tau**	**Aβ42**	**Aβ40**	**Tau**	**Aβ42**	**Aβ40**
Range (pg/mL)	0.853-2.19	8.02-13.4	153-250	0.287-1.14	8.07-12.8	144-238	19.0-4075	66.2-2217	1367-10749
Mean (pg/mL)	1.49	11.2	210	0.593	10.6	186	310	771	6555
Median (pg/mL)	1.42	11.1	209	0.561	10.6	183	82.5	592	6898

Fig. 4 . Test of Neurochem AD and Control plasma samples using Simoa Neurology 3–Plex assay. [Tau] in plasma (n = 38) is slightly higher in AD than control group (n=40). P=0.026. [Aβ42] is not different between AD and control. P= 0.39. [Aβ40] is higher in AD than control group, P=0.02. All three protein range overlaps between two groups. Error bars depict median and interquartile ranges.



CONCLUSIONS

The Simoa Human Neurology 3-plex assay is the first to reliably measure all 3 biomarkers simultaneously in plasma, serum and CSF samples from healthy individuals. It provides a new tool for the quantification of Aβ42, Aβ40 and total tau in both normal and diseased samples.

REFERENCES

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Corresponding author: Dr. L. Chang and Dr. D. Wilson
Quanterix Corporation 113 Hartwell Avenue, Lexington, MA 02421, USA
Tel: 1-617-301-9428
lchang@quanterix.com
dwilson@quanterix.com
www.quanterix.com