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Qualification of a Neurology 2-Plex B (Nf-L, GFAP) Quanterix Simoa® Assay Using a Novel Approach

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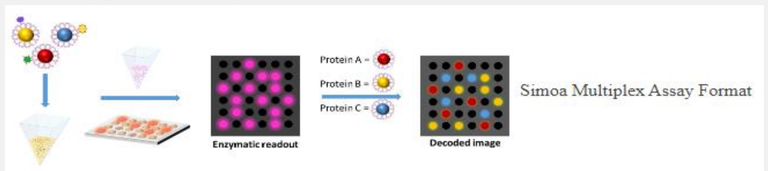
PURPOSE/OBJECTIVE

- Biomarkers associated with neurodegenerative diseases and traumatic brain injuries, including Nf-L (Neurofilament light) and GFAP (Glial fibrillary acidic protein) have been identified as potential prognostic tools.
- Decreases in GFAP have also been reported in Down's Syndrome, schizophrenia, bipolar disorder, and depression. Quanterix Neurology 2-Plex B (N2PB) Assay Kits measure both Nf-L and GFAP simultaneously in human serum, plasma, and CSF matrices in the fully automated Simoa® HD-X instrument.
- To qualify the method quantitative ranges in each matrix, high concentrations of proteins are required to achieve the necessary ranges, while maintaining the matrix integrity. However, obtaining reference material for both analytes with a high concentration to prepare controls in each matrix was not an option.
- **A novel approach was utilized when, during screening of individuals, a high endogenous concentration for both Nf-L and GFAP was observed in one human CSF individual. The CSF individual was used to prepare controls in human serum, plasma, and CSF to qualify the assay successfully on the Simoa® instrument**.

METHOD(S)

- Neurology 2-Plex B (N2PB) Assay kits were procured from Quanterix (Billerica, MA). Normal human serum and plasma were procured from BioIVT (Westbury, NY). Normal human cerebrospinal fluid (CSF) was procured from BioChemed (Winchester, VA).
- The method was conducted according to the manufacturer's recommendations and qualified according to current industry standards and regulatory guidance (FDA, 2018), (EMEA, 2011).
- A 3-step assay was performed autonomously in the HD-X analyzer, where a capture antibody coated bead was incubated with the sample in a cuvette. Target molecules (Nf-L, GFAP) that are present bind to the coated beads. After washing, biotinylated detector antibodies are added, mixed, and incubated with the beads. The detector antibodies will bind to the Nf-L and GFAP captured on the beads. After another wash, streptavidin-β-galactosidase (SBG) is mixed with the beads and binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured Nf-L and GFAP. Following a final wash, the beads are resuspended in a resorufin β-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa® disk.
- Individual beads are sealed within microwells in the array. If the target has been labeled and captured on the bead, SBG hydrolyzes the RGP substrate into a fluorescent product that will provide the signal (AEB, average enzymes per bead) for measurement.
- Back-calculated concentrations (BCC) of analyte are generated using an appropriate fit model (e.g., 4PL fit model),



Simoa Multiplex Assay Format

High CSF Indiv	Nf-L Fitted Conc (pg/mL)	Nf-L Adjusted Conc (pg/mL)	GFAP Fitted Conc (pg/mL)	GFAP Adjusted Conc (pg/mL)
1:100	317	31,700	7,906	790,600
1:150	219	32,850	5,733	859,950
1:250	113	28,250	3,266	816,500
	Ave	30,933	Ave	822,350
		92x Top Cal		94x Top Cal

Qualification Executive Summary of Results

Qualification Parameter		Biomarker Nf-L	Biomarker GFAP
Qualified Range: Calibrators	ULOQ (pg/mL)	336.00	8,733.00
	LLOQ (pg/mL)	0.318	8.47
Qualified Range: Plasma	ULOQ (pg/mL)	834.60	20,732.50
	LLOQ (pg/mL)	2.56	52.90
Qualified Range: Serum	ULOQ (pg/mL)	945.40	25,053.40
	LLOQ (pg/mL)	3.48	62.60
Qualified Range: CSF	ULOQ (pg/mL)	10,793.30	258,877.70
	LLOQ (pg/mL)	19.60	425.70
Selectivity	Serum	Pass	Pass
	Plasma	Pass	Pass
	CSF	Pass	Pass
Dilutional Linearity	Serum 1:2-1:162	Pass	Pass
	Plasma 1:2-1:162	Pass	Pass
	CSF 1:40-1:3,240	Pass	Pass
Short Term Stability: 24 hr. at 4°C	Serum	Pass	Pass
	Plasma	Pass	Pass
	CSF	Pass	Pass
Short Term Stability: 24 hr. at 22°C	Serum	Pass	Pass
	Plasma	Pass	Pass
	CSF	Pass	Pass
Freeze Thaw Stability: 5 Freeze/thaws	Serum	Pass	Pass
	Plasma	Pass	Pass
	CSF	Pass	Pass
Long Term Stability: 6 Months	Serum	Pass	Pass
	Plasma	Pass	Pass
	CSF	Pass	Pass

RESULT(S)

- A high endogenous concentration CSF individual was run at multiple dilutions on the HD-X instrument to measure the concentration of both Nf-L and GFAP to utilize it as the reference material for the assay's qualification.
- The qualified calibration curve range is from 0.318 to 336.00 pg/mL for Nf-L and from 8.47 to 8,733.00 pg/mL for GFAP. For human plasma, the qualified ranges are from 2.56 to 834.60 pg/mL for Nf-L and from 52.90 to 20,732.50 pg/mL for GFAP. For human serum, the qualified ranges are from 3.48 to 945.40 pg/mL for Nf-L and from 62.60 to 25,053.40 pg/mL for GFAP. For human CSF, the qualified ranges are from 19.60 to 10,793.30 pg/mL for Nf-L and from 425.70 to 258,877.70 pg/mL for GFAP.
- Selectivity was tested in 10 normal human serum and plasma individuals, and all passed acceptance criteria.
- Dilutional linearity was prepared by spiking the ULOQ concentration into 4 normal human serum and plasma individuals and diluting from 1/1-1/64, all samples measuring within the curve range passed acceptance criteria.
- Both analytes in human serum, plasma, and CSF were stable up to 24 hours at both room temperature and refrigerated. Both analytes in human serum, plasma, and CSF were stable up to 5 freeze/thaw cycles. Both analytes in human serum, plasma, and CSF were stable for up to 6 months for long term stability at -80°C.

CONCLUSION(S)

- A multianalyte method for the quantitation of Nf-L and GFAP in human serum, plasma, and CSF was qualified using a novel approach and meets current industry standards for all parameters evaluated.
- This method will provide data that may help discern subtle changes in responses to therapeutic treatments and improve diagnostic and prognostic efforts in various neurodegenerative diseases and following traumatic brain or spinal cord injuries.
- This method can help streamline clinical trials by providing decision makers with high-quality biomarker data which can lead to a more targeted and effective way of treating patients and improving their lives.

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