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Qualification of the S-PLEX Neurology Panel 1 (GFAP, NfL & Tau) MSD Assay

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PURPOSE

Neurofilament Light chain (NfL), glial fibrillary acidic protein (GFAP) and Tau have been identified as valuable diagnostic and prognostic biomarkers of brain injury and other neurological disorders[1]. In the context of a clinical trial, data from such measurements may play a critical role in informing treatment efficacy and safety evaluations, patient enrollments as well as stratification. Assays which evaluate these analytes in plasma and CSF can be used to measure changes in each matrix in response to treatment. Ultimately, this data can be used to support assessment of the biomarkers in plasma, which is a much less invasive sample to collect compared to CSF, in later phase clinical trials. Leveraging multiplexed panels for these biomarkers enables multiple analytes to be measured simultaneously from a single sample, which is especially advantageous for testing rare or volume limited samples. Platforms such as MSD offer a wide dynamic quantitation range and sensitivity with the added benefit of low sample volumes unlike conventional platforms such as ELISA.

OBJECTIVE

Here, we describe the qualification of an S-PLEX Neurology Panel 1 (NfL, Tau and GFAP) in, both, plasma and CSF matrices.

MATERIALS AND METHODS

- The Neurology Panel 1 was procured from Meso Scale Diagnostics (MSD, Cat# K15639S). Plasma and CSF from normal and Parkinson's Disease individuals were obtained from BioIVT.
- The assay was performed per manufacturer's instructions and qualified parameters included:
  - Accuracy & Precision
  - Parallelism
  - Stability and Ruggedness
- Ten normal donors were first evaluated to determine the endogenous levels of each of the 3 analytes in both plasma and CSF, and their placement on the standard curve, respectively. This not only aided in selecting the appropriate starting sample dilution factor for each of the analytes, but also in the preparation of 5 levels of endogenous qualification samples (ULOQ, HQC, MQC, LQC and LLOQ) in both matrices.

The S-PLEX method for the Neurology Panel 1 was qualified in plasma and CSF an MRD of 1:2 and 1:10, respectively. Quantification ranges are listed in **Table 1**. Representative calibration curves for all 3 analytes are shown in **Figure 1**.

Table 1: Quantification Ranges of Neurology Panel Analytes in Plasma and CSF

Analyte	Quantification Range (Plasma)	Quantification Range (CSF)
GFAP	479442 – 3016 fg/mL	5358116– 1822 fg/mL
NfL	5495822- 2469 fg/mL	31065114- 95316 fg/mL
Tau	225775- 242 fg/mL	1134236- 3952 fg/mL

Figure 1: Calibration Curves (GFAP, NfL, Tau) in Neurology S-Plex Panel 1

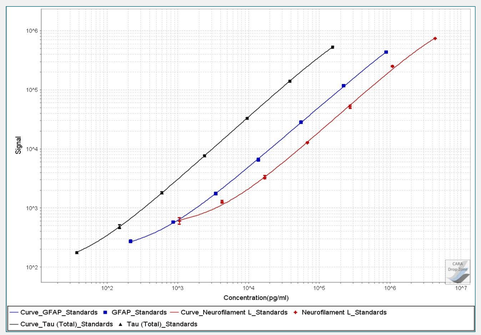


Figure 1: *Representative calibration curves from the S-Plex Neurology Panel 1. Standards for each analyte are depicted in the figure (1) GFAP in blue squares (2) NfL in red diamonds and (3) Tau in black triangles.*

All 3 analytes across both matrices demonstrated acceptable Accuracy & Precision (**Table 2**) and Parallelism (**Figure 2**), Short-term and Freeze-Thaw Stability (**Table 3**) as well as a lack of Prozone effect, except for GFAP, which failed 24-hour RT stability for plasma.

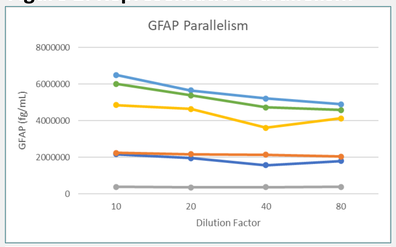
Table 2: Inter-Assay Precision & Accuracy

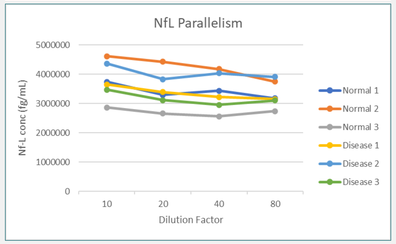
QS	PLASMA			CSF		
	GFAP Mean (fg/mL)	%CV	%RE	GFAP Mean (fg/mL)	%CV	%RE
QS1	305507.88	13.6	27.4	663010.69	14.0	23.7
QS2	48097.97	16.2	28.1	94792.03	7.1	15.8
QS3	9729.68	8.4	15.5	13588.97	1.9	5.4
QS4	1582.12	4.7	4.9	1819.86	13.5	-2.1
QS5	139.87	34.8	-45.4	195.64	9.9	7.4
	NfL Mean (fg/mL)	%CV	%RE	NfL Mean (fg/mL)	%CV	%RE
QS1	3173184.30	6.9	15.5	3344330.45	7.5	7.7
QS2	385632.65	5.0	12.2	446824.58	4.3	1.9
QS3	58784.82	3.0	3.7	66262.65	7.9	1.2
QS4	8499.40	14.5	-3.5	9038.25	14.8	-5.2
QS5	1416.29	15.6	14.7	1273.21	31.6	-1.3
	Tau Mean (fg/mL)	%CV	%RE	Tau Mean (fg/mL)	%CV	%RE
QS1	136046.3	9.8	20.5	128989.20	5.2	13.7
QS2	35773.7	7.4	16.8	18029.56	3.9	9.8
QS3	5739.467	3.7	10.5	2685.71	5.2	3.5
QS4	790.33	7.7	-1.6	362.70	12.5	-8.2
QS5	112.18	12.8	-7.4	53.31	32.1	-25.3

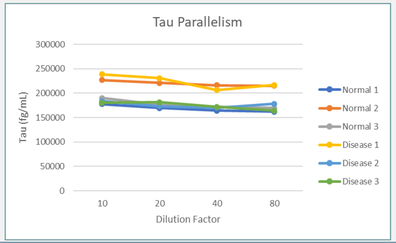
Table 3: Qualification Summary

Assessment	Matrix	GFAP	NfL	Tau
Short term stability @ 4ºC	CSF	PASS	PASS	PASS
	Plasma	PASS	PASS	PASS
Short term stability @ RT	CSF	PASS	PASS	PASS
	Plasma	DNP	PASS	PASS
Freeze-thaw stability (3X)	CSF	PASS	PASS	PASS
	Plasma	PASS	PASS	PASS
Prozone	CSF	PASS	PASS	PASS
	Plasma	PASS	PASS	PASS

Figure 2: Representative Parallelism







CONCLUSIONS

- The S-PLEX Neurology Panel 1 was successfully qualified in human CSF and plasma matrices.
- The qualified method would be applied in a clinical study to help explore the endogenous concentrations of GFAP, NfL and Tau in both the matrices.
- This assay combines 3 essential analytes for multiple neurological indications using the novel S-Plex methodology, demonstrating precision and accuracy to low fg/mL concentrations, which is a critical concern for these neural biomarkers where sample volume is extremely limited.

REFERENCE

1. Park Y, Kc N, Paneque A, Cole PD. Tau, Glial Fibrillary Acidic Protein, and Neurofilament Light Chain as Brain Protein Biomarkers in Cerebrospinal Fluid and Blood for Diagnosis of Neurobiological Diseases. Int J Mol Sci. 2024 Jun 7;25(12):6295.

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