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Validation of Ultra-high Sensitive SIMOA Assays for the Quantitation of Neurofilament Light Chain (NF-L) and Glial Fibrillary Acidic Protein (GFAP)

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PURPOSE

Neurodegenerative diseases affect reportedly fifty million people in the United States each year. There are over 600 types of neurodegenerative diseases, and the underlying causes can vary significantly. Over the past decade, biomarkers have played a significant role in diagnoses, prognoses, and clinical research. A series of neurodegenerative biomarkers have been identified and studied using various types of technologies. Blood-based biomarker testing provides a non-invasive tool to study neurodegenerative diseases. However, because low concentrations are a challenge, ultra-high sensitive assays are needed to support this testing. Biomarker analysis has progressed in two directions in the past few decades. One is focused on increasing the multiplexing capacity and the other is focused on improving the sensitivity. Quanterix SIMOA, for example, provides ultra-high sensitive detection and quantitation of a variety of neurodegenerative biomarkers such as neurofilament light chain (NF-L) and Glial Fibrillary Acidic Protein (GFAP). Herein, we report the validation of NF-L and GFAP in human plasma/serum and CSF using Quanterix SIMOA following FDA bioanalytical method validation guidance.

OBJECTIVE

Validation of NF-L and GFAP in human plasma/serum and CSF using Quanterix SIMOA following FDA's bioanalytical validation guidance.

METHODS

The assays use the commercial Simoa Advantage NF-Light and GFAP Discovery assay kits provided by Quanterix. For the methods, as the first step, anti-biomarker antibody-coated paramagnetic capture beads and an anti-biomarker antibody conjugated with biotin are incubated with samples diluted in sample diluent. Biomarker molecules present in the samples are captured by the antibody-coated capture beads. The detection antibody will then bind to the biomarker molecules. The beads are washed, and a streptavidin-ß-galactosidase (SßG) conjugate is mixed with the capture beads, where the SßG binds to the biotinylated detection antibody, resulting in the enzymatic labeling of the captured biomarker. Followed by wash steps, the capture beads are re-suspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc where the image is taken, and the data is analyzed.

RESULTS

Standard Curve

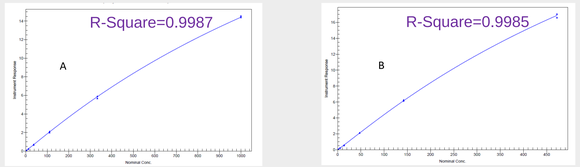

Figure 1. (A) Representative Standard Curve with 4PL Regression of Validated GFAP assay. The quantitative range of the GFAP method was 1.37–1000 pg/mL. (B) Representative Standard Curve with 4PL Regression of Validated NF-L assay. The quantitative range of the NF-L method was 0.673–472 pg/mL.

Accuracy and Precision

A

GFAP	Intra-assay Precision	Intra-assay Relative Error	Inter-assay Precision	Inter-assay Relative Error	% Total Error
ULOQ	3.3	7	6.2	6	12.2
HQC	2.2	11.3	6.2	4.3	10.5
MQC	6.6	-3.2	6.7	-3.5	10.2
LQC	4.6	-3.4	5.7	-0.6	6.3
LLOQ	4.2	5.7	9.6	0.7	10.3
EQC (Plasma)	6.7	3.2	5.4	0.7	6.1

B

GFAP	Intra-assay Precision	Intra-assay Relative Error	Inter-assay Precision	Inter-assay Relative Error	% Total Error
ULOQ	2.6	-5	8.4	3	11.4
HQC	3.9	-1.3	6.7	3.7	10.4
MQC	2.7	0.8	9.1	3.5	12.6
LQC	3	1.6	5.2	6.6	11.8
LLOQ	7.2	9.3	8	5	13
EQC (CSF)	5.1	-3	6.1	1.8	7.9

C

NF-L	Intra-assay Precision	Intra-assay Relative Error	Inter-assay Precision	Inter-assay Relative Error	% Total Error
ULOQ	4.1	-8.5	7.3	-8.9	16.2
HQC Plasma	2	2.2	8.8	-2.9	11.7
MQC Plasma	4.7	5.8	9	-4.7	13.7
HQC Serum	3.1	5.9	10.4	-4.9	15.3
MQC Serum	2.2	4.4	7.8	-4.9	12.7
HQC CSF	13.1	9.5	14.4	-5.8	20.2
MQC CSF	5.3	11.5	15.8	-7.7	23.5
LQC	6.8	16.7	15.3	-2.8	18.1
LLOQ	8.3	0.4	11.6	-5.1	16.7

Table 1. Accuracy and Precision of Validation of GFAP in Human Plasma (A), GFAP in Human CSF (B) and NF-L in Human Plasma, Human Scrum and Human CSF.

- All standards and validation samples were tested in duplicate wells.
- Parameters include standard calibration model, accuracy and precision, interference, detectability and reproducibility, dilution linearity, and stability (bench top at room temperature, refrigerator at 4°C, and freeze/thaw) and these have all been successfully validated.

Interference

Hemolysis of up to 5% hemolyzed blood and lipemic effect up to 500 mg/dL were also evaluated and no significant impact on the quantitation was observed.

	Hemolysis (up to 5% Hemolyzed Whole Blood)	Lipemic (up to 500 mg/dL)
GFAP	No Interference	No Interference
NF-L	No Interference	No Interference

Analyte Stability in Samples

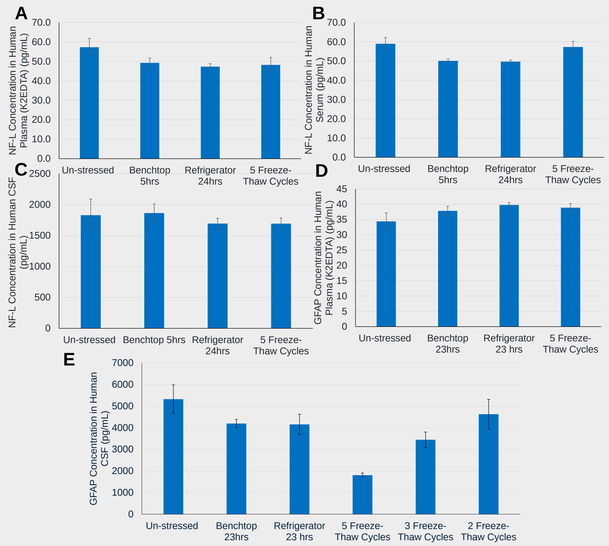

Figure 2. NF-L and GFAP Stability in samples. A-C: NF-L stability in human plasma (K2EDTA), Serum and CSF. D-E: GFAP stability in human plasma(K2EDTA) and CSF). All results are presented in Mean± SD (N=3).

Storage Stability-GFAP Unspecific Binding with Plastic

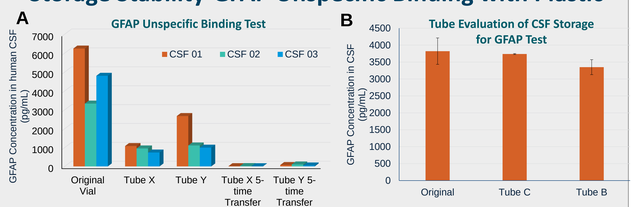

Figure 3. Unspecific Binding of GFAP in CSF. A: Transfer test of GFAP in different types of tube. B. Tube evaluation for GFAP validation (results are presented in Mean± SD (N=4)).

- Unspecific binding of GFAP in human plasma was observed during assay method development and validation.
- The detected GFAP concentration was reduced dramatically when using Tube X and Tube Y with one-time transfer and 5-time transfer tests.
- Tube evaluation test was performed using low-binding Tube B and Micro-tube C. With proper handling, the GFAP unspecific binding could be reduced and meet GLP lab testing requirements.

CONCLUSION

The validation data presented in this poster demonstrates that the following parameters met all acceptance criteria:

- Standard Calibration Model
- Accuracy and Precision
- Interference
- Detectability and Reproducibility
- Dilution Linearity
- Stability (bench top at room temperature, refrigerator at 4°C, and freeze/thaw)

Both NF-L and GFAP are highly reliable, reproducible and are used for supporting high sensitivity biomarker profiling and analysis.

FUNDING/GRANT/ENCORE/REFERENCE OR OTHER USE

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