COMPANION DIAGNOSTICS FOR ALZHEIMER DISEASE

Neurofilament Light Chain: Method Validation of Quantitation Using the Quanterix Simoa™ Platform and Potential Applications

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ALZHEIMER DISEASE AND ASSOCIATED PROTEIN BIOMARKERS

- Alzheimer disease impact over 3 million families across the United States each year. Despite there has no effective treatment by far, various pipelines of clinical trials being conducted all over the world to tackle this disease.
- Companion Diagnostic is using biomarker to monitor and predict patient response toward drug.
- Patients with Alzheimer disease experienced brain cell damage and eventually loses memories and other mental functionalities. Various biomarkers are identified (Table a).
- Typical platforms for biomarkers analysis listed in Table b.
- Based on possible pathological processes, Amyloid beta and Tau proteins work together, to form plaques and tangles, respectively, and leads to the neuron degeneration.
- Because the toxic aggregation of both Amyloid beta and Tau proteins can spread to the brain gradually, an early diagnostic and intervention is important.
- Recent research indicates phospho-Tau 217, 181 serves as excellent biomarker for Alzheimer disease. Phospho-Tau 217 gave higher dynamic range than p-Tau 181, in comparison with normal healthy individuals, thus, serves as potential better biomarker than phospho-Tau 181, for patient enrichment and companion diagnostic.
- Neurofilament Light Chain (NF-L) is a debris protein from axonal damage that is presented in human blood and CSF.
- NFL concentration increases over the course of disease progression, thus, served as a biomarker of neurodegeneration for Alzheimer disease.

Table a. Common Alzheimer Disease Biomarkers

Panel Biomarker	Blood-based Biomarker	Comments
Genomic biomarkers	APOE e4 Allele PSEN1, PSEN2, etc.	Combined Genetic Risk Score (GRSs)
Protein biomarkers	Neurofilament light (NFL), t-Tau/p-Tau, Aβ40/42	Amyloid Beta 42 peptide and Tau protein correlation Serum NFL is highly correlated with CSF levels
Metabolic biomarkers	Sodium (Na), Glucose levels, Biocarbonate (HCO3), High creatinine (Cr), Blood-based lipid panel, etc.	Electrolytes (comprehensive metabolic panel) A set of 10 phospholipids (10-lipid panel)
Others	CBC (complete blood count) THS(Thyroid stimulating hormone)/T4 Vitamin B12 RPR (rapid plasma regain), HIV (human immunodeficiency virus) Liver function tests (LEFs) mRNA signature, etc.	Anemia, infection Thyroid disorder B12 deficiency Screening test for syphilis HIV can cause changes in thinking or memory Etc.

CASE STUDY: NF-L ASSAY

- Herein, we report quantitation of NF-L in human plasma, serum and CSF on the Quanterix Simoa Platform using commercial Simoa NF-Light Assay Kit provided by Quanterix Corporation.

Sample preparation: an anti-NF-L antibody conjugated with Biotin and ananti-NF-L antibody-coated paramagnetic capture beads are mixed with study sample and sample diluent for incubation. NF-L are captured by the anti-NF-L antibody-coated capture beads. The NF-L molecule will then be sandwiched by the detection antibody. After washing, a streptavidin-ß-galactosidase (SßG) conjugate is mixed with the capture beads. SßG binds to the biotinylated detection antibody, enabling the enzymatic labeling of NF-L molecules. After one additional wash step, the capture beads are re-suspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc, which are subsequently sealed within microwells in the array.

Detection and data aquisition: After NF-L molecule is captured, the RGP substrate will be hydrolyzed by ß-galactosidase species for fluorescent detection and measurement. The signal will be detected by the Simoa optical system. A “digital” and “analog” signal is captured when the NF-L concentration is at the lower and higher end of the calibration curve, respectively. The actual NF-L concentration in study samples will be back calculated based on the calibration curve.

Instrument and computer system: Simoa HD-1/HD-X analyzer was used to capture raw data. Watson LIMS version 7.6 handled the data management and calculation of the validation sample concentrations. Watson LIMS and Microsoft® Office Excel were used for statistical calculations. When Microsoft® Office Excel was used, the calculations were 100% audited.

Calibration standards and QC samples: Calibration standards were prepared in assay buffer. QC samples were prepared in buffer (LLOQ, ULOQ and LQC) and authentic matrix (MQC and HQC). The concentrations are listed in Table c.

Acceptance criteria, general: Accuracy (%Bias) and precision (%CV) within 20.0% (25.0% at LLOQ).

Table b. Biomarker comprehensive suit of platforms

ICP-MS for trace elements as the biomarkers
GC-MS for small molecule biomarkers
LC-MS/MS for small molecule and protein biomarkers
ELISA Assay Kits (single analyte)
Meso Scale Discovery (single or multiplex)
Quanterix Simoa™ HD-1, HD-X, SP-X (single or multiplex, fg/mL sensitivity)
Ella Protein Simple System (single or multiplex, high throughput)
Magpix Luminex (multiplex, biomarker screening)
Gyros Workstation (nano-liter sample volume, large dynamic range)
qPCR, ddPCR Platform, NGS
Flow Cytometry

Table c. QC Type and concentrations

Quality Control	Concentration (pg/mL)
ULOQ (Buffer)	472
*HQC (Plasma)	903
*MQC (Plasma)	57.3
*HQC (Serum)	1020
*MQC (Serum)	59.0
*HQC (CSF)	32800
*MQC (CSF)	1830
LQC (Buffer)	4.32
LLOQ (Buffer)	0.673

Note: *Matrix QC concentrations were determined from the first two inter-A&P runs and the intra-A&P run.

CASE STUDY: NF-L ASSAY, VALIDATION RESULTS

LLOQ: 0.673 pg/mL (26.9 pg/mL, adjusted for human plasma and serum; 67.3 pg/mL, adjusted for human CSF)

ULOQ: 472 pg/mL (1890 pg/mL, adjusted for human plasma and serum; 47200 pg/mL, adjusted for human CSF)

Dilution: No dilution for standards; 1:4 for QC1/2, Plasma QCs/samples, and serum QCs/samples; 1:100 for CSF QCs/samples

Accuracy and Precision: Refer to Table d

Detectability: 90% (9 out of 10) of normal human CSF samples, 100% (10 out of 10) of normal human serum samples and 100% (10 out of 10) of normal human plasma samples met the acceptance criteria.

Table d. Intra- and Inter- Accuracy and Precision

QC Concentrations (pg/mL)	Intra-assay Precision (%CV)	Intra-assay Relative Error (%RE)	Inter-assay Precision (%CV)	Inter-assay Relative Error (%RE)
ULOQ	4.1	-8.5	7.3	-8.9
HQC Plasma	2.0	2.2	8.8	-2.9
MQC Plasma	4.7	5.8	9.0	-4.7
HQC Serum	3.1	5.9	10.4	-4.9
MQC Serum	2.2	4.4	7.8	-4.9
HQC CSF	13.1	9.5	14.4	-5.8
MQC CSF	5.3	11.5	15.8	-7.7
LQC	6.8	16.7	15.3	-2.8
LLOQ	8.3	0.4	11.6	-5.1

COMPANION DIAGNOSTIC AND EARLY DIAGNOSIS

- Companion diagnostic is crucial in drug development and is able to help predict patient response toward drug.
- NF-L, as one of the important biomarker for neurodegenerative-related diseases including Alzheimer disease and multiple sclerosis. To utilize NF-L as a tool for companion diagnostic purpose, a specific and sensitive assay is necessary. Quanterix, developed the selective antibody pair for NF-L assay kits and with the SIMOA technology, we validated the ultra sensitive assay. Based on the detectability of NF-L from samples from healthy donors, this assay is sensitive enough to quantitate the NF-L for normal population, and will also be able to detect NF-L level in diseased population considering the expected increase of NF-L level over the progression of neuron degeneration of patients.
- By utilizing the NF-L assay on clinical trials, a correlation of drug treatment against NF-L level can be studied. And NF-L can be used as a secondary or even primary end-point of clinical trial.
- Potentially, the assay can be used as a standardized tool for the industry for Alzheimer disease diagnosis.
- Multiple Tau proteins, including phospho-Tau 181, phospho-Tau 217 and total Tau, also has the potential to server as a biomarker for companion diagnostic purpose.
- A sensitive biomarker assay such as NF-L assay, is able to serve as a non-invasive tool for early diagnosis.
- Early diagnosis for Alzheimer disease is very important. Early intervention could potentially improve cognitive functions of patients.
- Early diagnosis could also assist patient selection and predictive enrichment of patients.

CONCLUSIONS

- A SIMOA assay for quantitation of NF-L biomarker was validated in human plasma, serum and CSF.
- The method is highly selective, ultra-sensitive and is able to detect NF-L levels for both healthy population and diseased population.
- These types of sensitive assays can assist early diagnostic as well as serving as a tool for companion diagnostic during drug development.