Quanterix™
The Science of Precision Health

Simoa™ N4PA Advantage Kit
SR-X Data Sheet

Item 102153

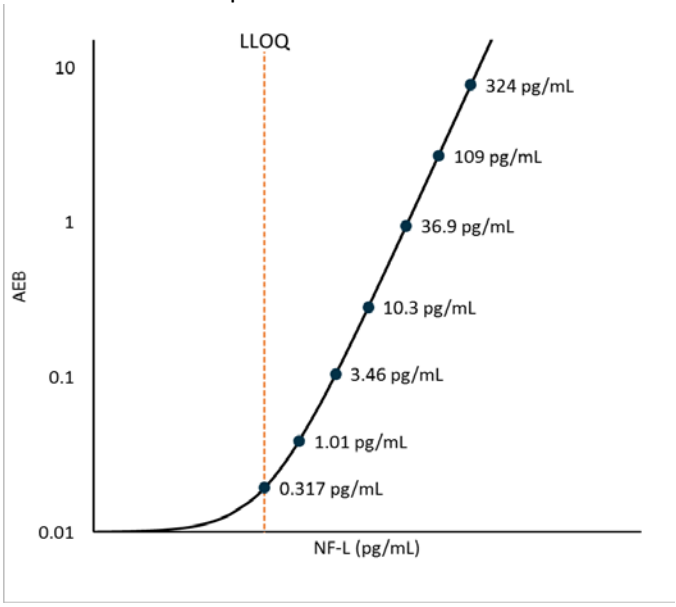
Description – Human Nerology 4-Plex "A"

The Simoa Human Neurology 4-Plex A assay (N4PA) measures four important neurology biomarkers in both cerebrospinal fluid (CSF) and blood. The four targets are neurofilament light (NF-L), total tau, glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). All four biomarkers have been studied as indicators of traumatic brain injury (TBI) severity. Recent reports indicate serum NF-L is a biomarker for mild TBI in amateur boxers and professional hockey players[1], that plasma tau is related to concussion severity[2], and that serum GFAP and UCH-L1 can detect mild to moderate TBI.[3]

Description – NF-light Test

Neurofilament light (NF-L) is a 68 kDa cytoskeletal intermediate filament protein that is expressed in neurons. It associates with the 125 kDa Neurofilament medium (NF-M) and the 200 kDa Neurofilament heavy (NF-H) to form neurofilaments. They are major components of the neuronal cytoskeleton, and are believed to function primarily to provide structural support for the axon and to regulate axon diameter. Neurofilaments can be released in significant quantity following axonal damage or neuronal degeneration. NF-L has been shown to associate with traumatic brain injury, multiple sclerosis, frontotemporal dementia and other neurodegenerative diseases. The Simoa NF-light® assay is a digital immunoassay for the quantitative determination of NF-L in serum, plasma and CSF. The antibodies (Uman Diagnostics, Umeå Sweden) also cross react with murine, bovine, and macaque NF-L epitopes, and the assay can be used for research with these species.

NF-L Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – GFAP Test

Glial Fibrillary Acidic Protein (GFAP) is a class-III intermediate filament majorly expressed in astrocytic glial cells in the central nervous system. Astrocytes play a variety of key roles in supporting, guiding, nurturing, and signaling neuronal architecture and activity. Monomeric GFAP is about 55kD. It is capable of forming both homodimers and heterodimers; GFAP can polymerize with other type III proteins or with neurofilament protein (such as NF-L). GFAP is involved in many important CNS processes, including cell communication and the functioning of the blood brain barrier. GFAP, as a potential biomarker has been shown to associate with multiple diseases such as traumatic brain injury, stroke, brain tumors, etc. Decreases in GFAP expression have been reported in Down's syndrome, schizophrenia, bipolar disorder, and depression.

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113 Hartwell Avenue, Lexington, MA 02421
techsupport@quanterix.com

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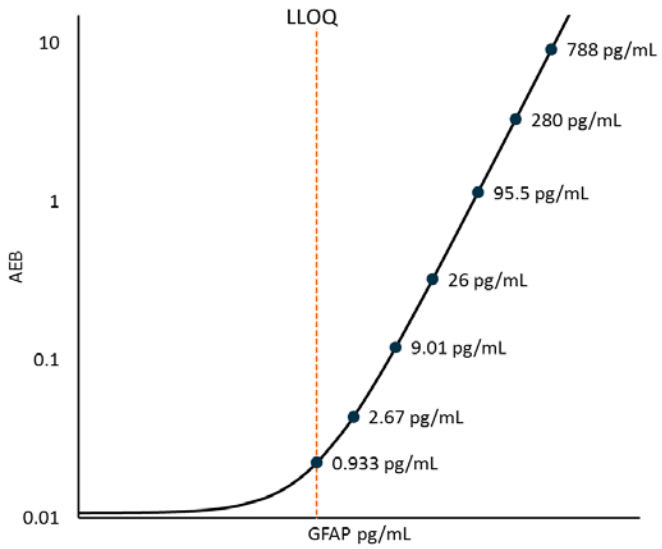
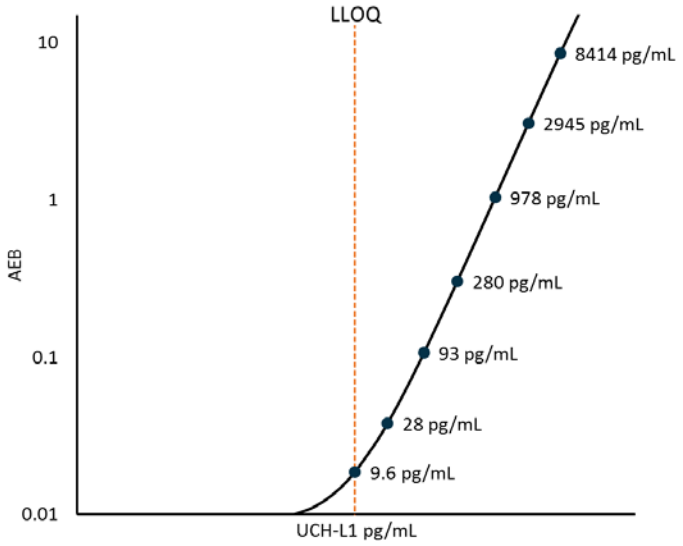
GFAP Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – UCH-L1 Test

The Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), hydrolyzes small C-terminal adducts of ubiquitin to generate the ubiquitin monomer. It is also called PARK5 or neuronal-specific protein gene product 9.5. Expressed predominantly in neurons, UCH-L1 is one of the most abundant brain proteins, representing 1 to 2% of total soluble brain protein. In vivo, UCH-L1 has been shown to be involved in the regulation of the ubiquitin pool, apoptosis, and learning and memory. Its absence in mice due to spontaneous intragenic deletions yields phenotypes with neurological defects. A point mutation (I93M) and a polymorphism (S18Y) in this gene have been shown to associate with Parkinson's disease. Recently, UCH-L1 has been proposed as a candidate biomarker for brain injury. UCH-L1 can be released from injured neurons and flow into the cerebrospinal fluid and circulating blood.

UCH-L1 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – Tau Test

Tau is a microtubule-stabilizing protein primarily localized in central nervous system neurons, but also expressed at low levels in astrocytes and oligodendrocytes. Tau consists of six isoforms in the human brain, with molecular weights of 48,000 to 67,000 daltons depending on isoform. Tau elevation is observed in the cerebrospinal fluid (CSF) of patients with neurodegenerative disease and head injuries, suggesting its extracellular release during neuronal damage and a role as a biomarker with specificity for brain injury. Potential movement of elevated CSF tau across the blood-brain barrier presents a possibility that measurements of tau in blood could provide a convenient peripheral window into brain/CSF status. Studies of tau in serum and plasma have been hampered by its low abundance (typically low pg/mL), and there are relatively few reports characterizing the appearance of tau in blood or evaluating the usefulness of this biomarker for brain injury assessment. Recent reports using digital immunoassay technology have shown elevation in peripheral tau associated with hypoxic brain injury, concussed hockey players, and repetitive minimal head injury in Olympic boxing. The Simoa™ Human Neurology 4-Plex Total Tau assay uses a combination of monoclonal antibodies that react with both normal and phosphorylated tau. With an epitope in the midregion of the molecule, the assay recognizes all tau isoforms.

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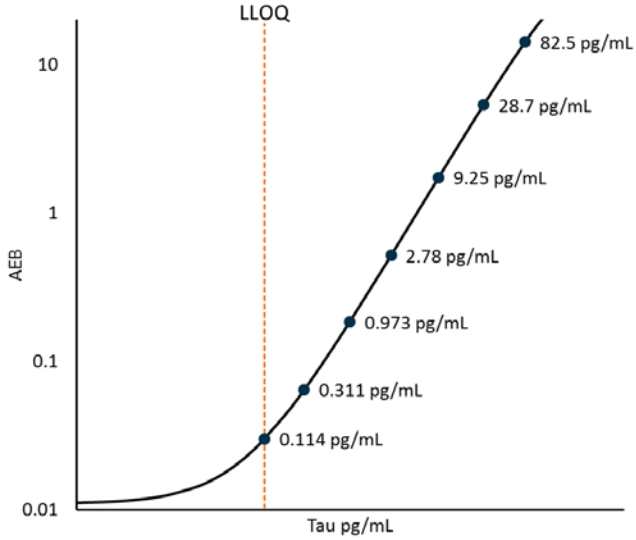
Tau Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

NF-L LLOQ	**0.317 pg/mL** pooled CV 18% mean recovery 101%
GFAP LLOQ	**0.933 pg/mL** pooled CV 20% mean recovery 93%
UCH-L1 LLOQ	**9.60 pg/mL** pooled CV 19% mean recovery 97%
Tau LLOQ	**0.114 pg/mL** pooled CV 16% mean recovery 94%

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

NF-L LOD	**0.136 pg/mL** range 0.054-0.277 pg/mL
GFAP LOD	**0.276 pg/mL** range 0.119-0.400 pg/mL
UCH-L1 LOD	**4.03 pg/mL** range 2.35-5.52 pg/mL
Tau LOD	**0.0298 pg/mL** range 0.0110-0.0464 pg/mL

Sample Testing: The ranges listed below are for serum and EDTA plasma. The ULOQ for CSF is 10X the ULOQ for serum and plasma.

NF-L Dynamic range	0- ~2000 pg/mL
GFAP Dynamic range	0- ~4000 pg/mL
UCH-L1 Dynamic range	0- ~40000 pg/mL
Tau Dynamic range	0- ~400 pg/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

***Serum and Plasma diluted 1:4 and CSF diluted 1:40. See Kit Instruction for details**

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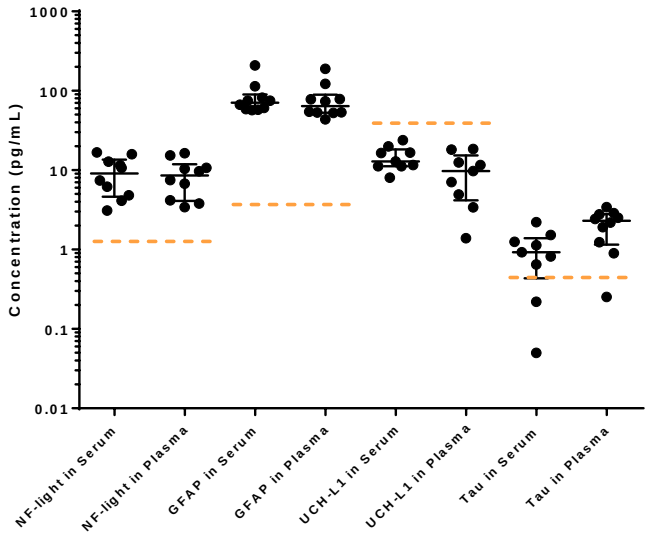
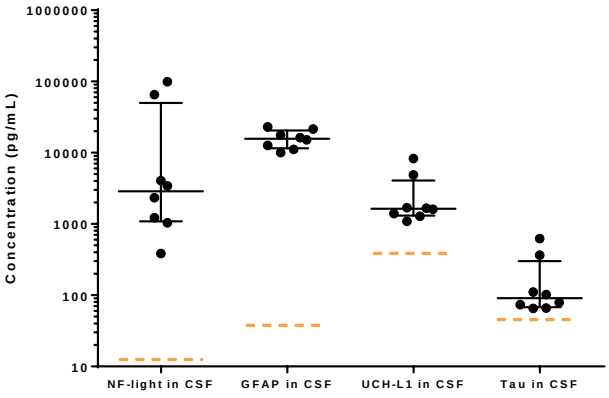
Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples were measured. Six UCH-L1 serum samples, eight UCH-L1 plasma samples, and two Tau serum samples could not be detected. These undetectable samples were excluded from the mean concentration but included in the median concentration assessments. Bars depict median with interquartile range. Orange lines represent functional LLOQ.



Endogenous CSF Readings: CSF (n=10) samples were measured. Two samples read above all four target ranges. Bars depict median with interquartile range. Orange lines represent functional LLOQ.



Target	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD
NF-L	EDTA plasma	8.81	8.58	100%
	Serum	9.32	9.07	100%
	CSF	22073	2874	100%
GFAP	EDTA plasma	79.9	64.2	100%
	Serum	85.6	70.7	100%
	CSF	15913	15692	100%
UCH-L1	EDTA plasma	*	*	20%
	Serum	*	*	40%
	CSF	2734	1635	100%
Tau	EDTA plasma	2.25	2.31	100%
	Serum	1.21†	0.926	80%
	CSF	186	90.6	100%

*No values above LLOQ
†Values below LLOQ are not included in the mean

Precision: Measurements of 3 serum or plasma based panels and 2 calibrator based controls. Triplicate measurements were made for 3 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements).

NF-L	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	4.56	7.1%	8.0%	1.7%
Control 2	330	4.9%	2.7%	0.9%
Panel 1	17.9	4.4%	4.3%	1.8%
Panel 2	64.8	3.7%	3.5%	2.3%
Panel 3	336	2.5%	3.5%	1.1%

GFAP	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	21.6	7.9%	5.6%	1.3%
Control 2	864	9.1%	7.5%	2.5%
Panel 1	63.8	9.9%	6.2%	2.4%
Panel 2	222	8.8%	4.0%	1.4%
Panel 3	1614	5.1%	6.6%	2.6%

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UCH-L1	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	86.6	10.4%	7.9%	1.2%
Control 2	6273	4.3%	2.6%	1.7%
Panel 1	12.6	31.8%	24.4%	7.1%
Panel 2	317	5.0%	6.2%	3.1%
Panel 3	5356	4.5%	3.7%	2.8%

Tau	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	2.00	3.7%	4.0%	2.0%
Control 2	80.3	2.8%	2.5%	1.2%
Panel 1	2.05	6.5%	4.6%	0.2%
Panel 2	17.2	3.6%	4.1%	3.3%
Panel 3	136	3.4%	3.9%	1.3%

Note: Data in the following sections were obtained using the HD-1 Analyzer.

Spike and Recovery, Serum and Plasma: 3 EDTA plasma samples and 3 serum samples, and 4 CSF samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

NF-L	**90%** Range 68-126%
GFAP	**105%** Range: 54-146%
UCH-L1	**95%** Range: 58-131%
Tau	**118%** Range: 52-193%

Spike and Recovery, CSF: 4 CSF samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

NF-L	**108%** Range 89-125%
GFAP	**119%** Range: 88-177%
UCH-L1	**119%** Range: 96-167%
Tau	**116%** Range: 97-138%

Dilution Linearity, Serum: 1 spiked serum sample was diluted 2X serially from 4x (MRD) to 512x with Sample Diluent.

NF-L (512x)	**Mean = 119%** Range: 101-155%
GFAP (512x)	**Mean = 108%** Range: 102-121%
UCH-L1 (512x)	**Mean = 90%** Range: 79-103%
Tau (512x)	**Mean = 115%** Range: 106-126%

Dilution Linearity, CSF: 1 spiked CSF sample was diluted 2X serially from 40x (MRD) to 5120x with Sample Diluent.

NF-L (5120x)	**Mean = 100%** Range: 93-110%
GFAP (5120x)	**Mean = 98%** Range: 96-104%
UCH-L1 (5120x)	**Mean = 96%** Range: 94-98%
Tau (5120x)	**Mean = 92%** Range: 78-121%

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The Simoa N4PA assay kit is formulated for use on either the SR-X or HD-1 platform. Minor differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two different platforms, due to experiments run at different time-points with different reagent lots and different samples. Data in this document were obtained from runs on the SR-X platform unless otherwise noted.

References

1 Shahim P, Zetterberg H, Tegner Y, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. Neurology 2017; (e-version ahead of print).

2 Gill J, Merchant-Borna K, Jeromin A, Livingston W, Bazarian J. Acute plasma tau relates to prolonged return to play after concussion. Neurology. 2017: 7;88(6):595–602.

3 Papa L, Brophy GM, Welch RD, Lewis LM, Braga CF, Ciara NT, et al. Time course of diagnostic accuracy of glial and neuronal blood biomarkers GFAP and UCH-L1 in a large cohort of trauma patient with and without mild traumatic brain injury. JAMA Neurol 2016; 73(5):551–60.

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