Quanterix
Discovery Fueled by Ultra-Sensitivity

Simoa® N4PA Advantage Kit
HD-1/HD-X Data Sheet

Item 102153

Description – Human Neurology 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, that plasma tau is related to concussion severity, and that serum GFAP and UCH-L1 can detect mild to moderate TBI.

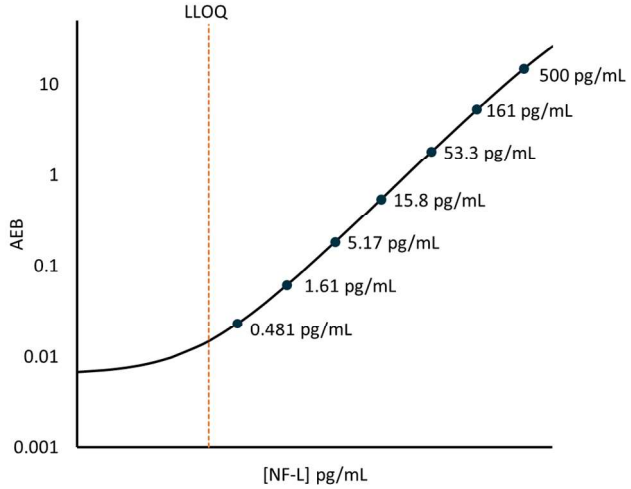
Description – NF-light Test

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.

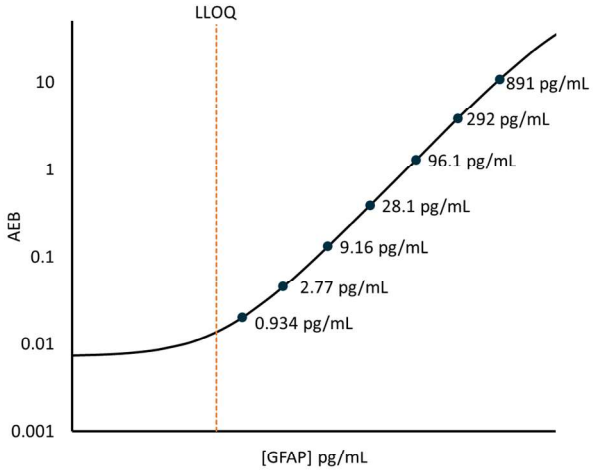
Description – GFAP Test

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.

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



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



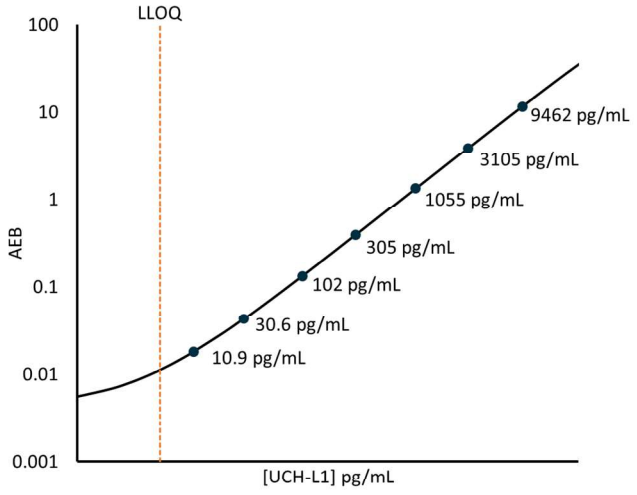
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Description – UCH-L1 Test

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.

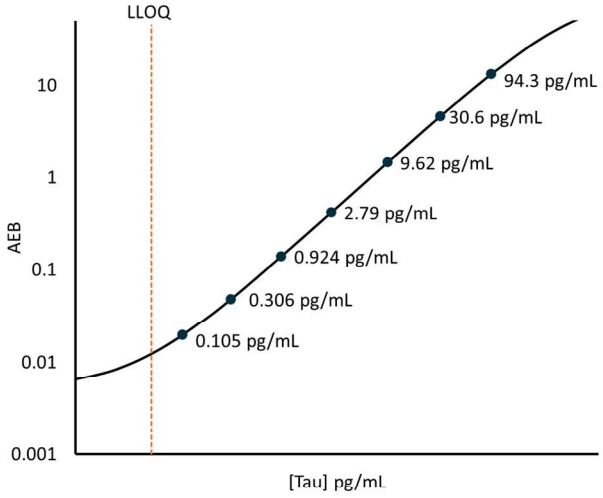
UCH-L1 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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.

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



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Minimal Required Dilution (MRD)

Diluted Sample Volume	152 µL per measurement
Serum and Plasma Dilution	1:4
CSF Dilution	1:40

See Kit Instructions for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve for 2 reagent lots across 3 instruments (12 runs total). The functional LLOQ (fLLOQ) values below are for serum and plasma. The fLLOQ for CSF is 10X the fLLOQ for serum and plasma.

	Analytical LLOQ	Functional LLOQ (x MRD)
NF-light	**0.241 pg/mL** pooled CV 12.0% mean recovery 110%	Serum/Plasma: **0.964 pg/mL** CSF: **9.64 pg/mL**
GFAP	**0.467 pg/mL** pooled CV 12.9% mean recovery 103%	Serum/Plasma: **1.87 pg/mL** CSF: **18.7 pg/mL**
UCH-L1	**5.45 pg/mL** pooled CV 20% mean recovery 115%	Serum/Plasma: **21.8 pg/mL** CSF: **218 pg/mL**
Tau	**0.053 pg/mL** pooled CV 12.2% mean recovery 109%	Serum/Plasma: **0.212 pg/mL** CSF: **2.12 pg/mL**

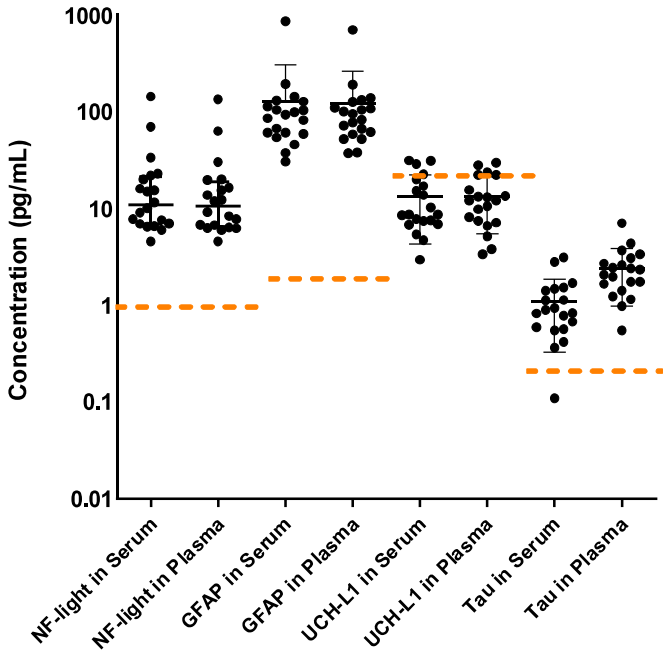
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve for 2 reagent lots across 3 instruments (12 runs total).

	LOD
NF-light	**0.104 pg/mL** range 0.025-0.276 pg/mL
GFAP	**0.221 pg/mL** range 0.042-0.481 pg/mL
UCH-L1	**1.74 pg/mL** range 0.120-3.62 pg/mL
Tau	**0.024 pg/mL** range 0.007-0.059 pg/mL

Assay Range: The ranges listed below are for serum and EDTA plasma. The Upper Limit of Quantification (ULOQ) for CSF is 10x the ULOQ for serum and plasma.

	Assay Range
NF-light	0-2000 pg/mL
GFAP	0-4000 pg/mL
UCH-L1	0-40000 pg/mL
Tau	0-400 pg/mL

Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma, and serum were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



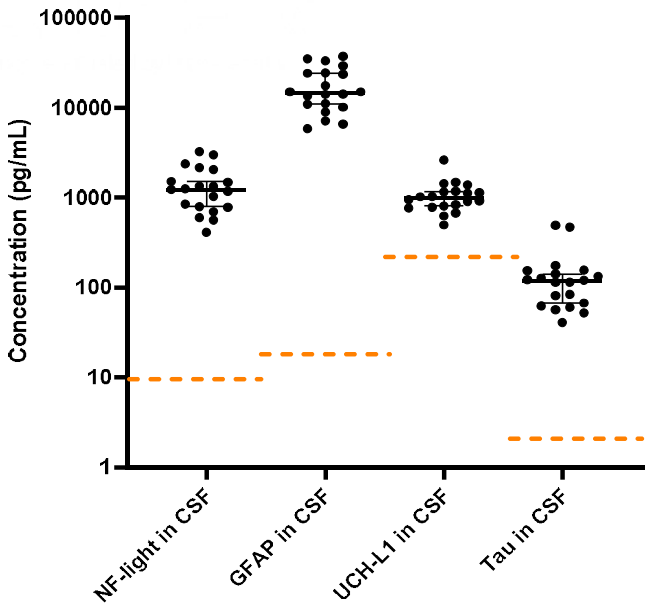
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Endogenous CSF Readings: Healthy donor CSF (n=20) samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD	% Above LLOQ
NF-light	Plasma	20.4	10.6	100%	100%
	Serum	22.2	10.8	100%	100%
	CSF	1400	1241	100%	100%
GFAP	Plasma	121	89.7	100%	100%
	Serum	129	90.2	100%	100%
	CSF	17923	14624	100%	100%
UCH-L1	Plasma	25.3	23.7	90%	0%
	Serum	28.6	30.2	90%	0%
	CSF	1069	989	100%	100%
Tau	Plasma	2.43	2.21	100%	100%
	Serum	1.14	0.891	100%	100%
	CSF	142	118	100%	100%

Values below LLOQ are not included in the mean or median.

*No values above LLOQ

Reproducibility Precision: Measurements of 3 serum- or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 12 runs each for 2 reagent lots across 3 instruments.

NF-L	Mean (pg/mL)	Between inst CV	Between lot CV	Between run CV	Within run CV
Control 1	9.31	0.9%	3.4%	5.8%	6.2%
Control 2	925	0.0%	6.1%	4.6%	4.4%
Panel 1	16.8	0.0%	0.0%	5.1%	5.4%
Panel 2	60.1	0.0%	0.0%	5.6%	4.7%
Panel 3	332	0.0%	3.7%	5.0%	4.7%

GFAP	Mean (pg/mL)	Between inst CV	Between lot CV	Between run CV	Within run CV
Control 1	27.6	0.0%	3.9%	3.0%	7.7%
Control 2	1492	0.0%	2.2%	7.1%	4.2%
Panel 1	59.4	0.0%	8.5%	3.8%	3.9%
Panel 2	226	0.0%	3.0%	6.2%	3.6%
Panel 3	2095	0.0%	6.5%	3.8%	4.5%

UCH-L1	Mean (pg/mL)	Between inst CV	Between lot CV	Between run CV	Within run CV
Control 1	129	1.8%	8.0%	2.9%	9.3%
Control 2	13145	0.0%	2.7%	4.9%	5.4%
Panel 1	71.9	5.0%	4.3%	6.4%	11.3%
Panel 2	381	4.8%	3.5%	4.9%	5.6%
Panel 3	6663	0.0%	3.5%	5.9%	4.2%

Tau	Mean (pg/mL)	Between inst CV	Between lot CV	Between run CV	Within run CV
Control 1	3.71	7.1%	2.6%	1.6%	7.6%
Control 2	200	0.0%	6.7%	4.5%	4.5%
Panel 1	2.66	0.0%	0.0%	5.7%	6.7%
Panel 2	20.2	1.5%	0.0%	6.6%	4.9%
Panel 3	148	5.2%	0.0%	3.0%	5.2%

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Repeatability Precision: Measurements of 3 serum- or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 10 runs at two separate times per day for five days using one lot of reagents.

NF-L	Mean (pg/mL)	Between day CV	Between run CV	Within run CV
Control 1	8.74	0.0%	6.5%	6.1%
Control 2	852	2.5%	0.0%	4.3%
Panel 1	16.1	0.0%	4.4%	6.0%
Panel 2	61.5	0.0%	0.0%	4.6%
Panel 3	325	0.0%	7.8%	4.5%

GFAP	Mean (pg/mL)	Between day CV	Between run CV	Within run CV
Control 1	26.2	2.7%	0.0%	7.2%
Control 2	1372	5.4%	3.1%	4.5%
Panel 1	60.5	2.6%	2.0%	5.6%
Panel 2	221	0.0%	4.0%	4.2%
Panel 3	1910	0.0%	8.5%	5.0%

UCH-L1	Mean (pg/mL)	Between day CV	Between run CV	Within run CV
Control 1	125	0.0%	2.8%	6.9%
Control 2	11681	3.6%	3.1%	4.0%
Panel 1	73.0	0.0%	10.6%	9.6%
Panel 2	356	4.0%	6.7%	5.6%
Panel 3	6082	0.0%	7.2%	4.2%

Tau	Mean (pg/mL)	Between day CV	Between run CV	Within run CV
Control 1	3.55	0.0%	6.0%	6.3%
Control 2	183	0.0%	4.6%	4.8%
Panel 1	2.66	0.0%	12.4%	7.4%
Panel 2	20.6	0.0%	7.6%	4.3%
Panel 3	142	0.0%	6.2%	4.3%

Spike and Recovery, Serum and Plasma: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

	Recovery
NF-light	**67.5%** Range 54.4-85.1%
GFAP	**77.2%** Range: 49.3-120%
UCH-L1	**107%** Range: 87.3-125%
Tau	**113%** Range: 76.7-158%

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.

	Recovery
NF-light	**108%** Range 88.8-125%
GFAP	**119%** Range: 87.5-177%
UCH-L1	**119%** Range: 96.0-167%
Tau	**116%** Range: 96.3-138%

Dilution Linearity, Serum: Spiked serum diluted 2x serially from MRD (4x) to 128x with Sample Diluent.

	Linearity
NF-light	**Mean = 119%** Range: 101-155%
GFAP	**Mean = 108%** Range: 102-121%
UCH-L1	**Mean = 90.1%** Range: 79.0-103%
Tau	**Mean = 115%** Range: 106-126%

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Dilution Linearity, CSF: CSF diluted 2x serially from MRD (40x) to 1280x with Sample Diluent.

	Linearity
NF-light	**Mean = 99.9%** Range: 92.6-110%
GFAP	**Mean = 98.1%** Range: 95.5-104%
UCH-L1	**Mean = 95.8%** Range: 93.5-98.0%
Tau	**Mean = 91.6%** Range: 78.1-121%

Admixture Linearity: High serum sample fractionally admixed with low serum sample, mean of 10 levels.

	Linearity
NF-light	**Mean = 94.9%** Range: 87.6-101%
GFAP	**Mean = 101%** Range: 88.9-112%
UCH-L1	**Mean = 95.4%** Range: 85.1-104%
Tau	**Mean = 94.3%** Range: 83.4-106%

The Simoa N4PA Advantage assay kit is formulated for use on the SR-X®, HD-1, or HD-X® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.

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