Simoa® Neurology 4-Plex B Kit
HD-1/HD-X Data Sheet Item 103345

Human Neurology 4-Plex "B"

The Simoa Human Neurology 4-Plex B assay (N4PB) 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. The Simoa Human Neurology 4-Plex A assay (N4PA) is also designed to measure the same 4 analytes. In samples from patients with severe TBI, levels of GFAP often saturate signal in the N4PA assay. N4PB has been developed to generate 10X lower signal for GFAP while retaining the ability to measure GFAP in most samples from healthy individuals. This modification results in better data yield from sample sets with high GFAP levels.

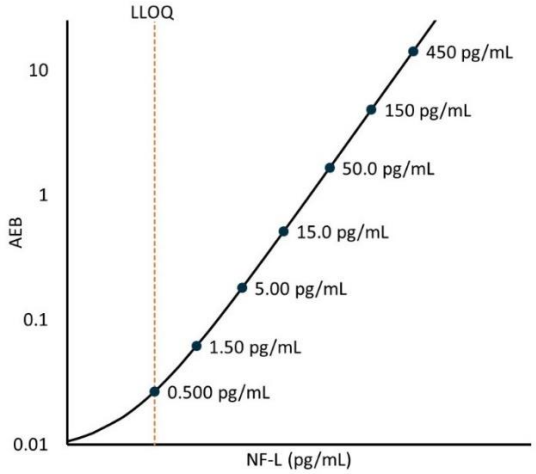
Description – NF-light Test

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.

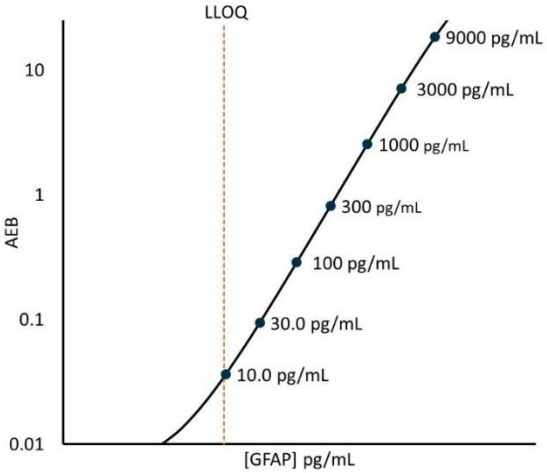
Description – GFAP Test

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.



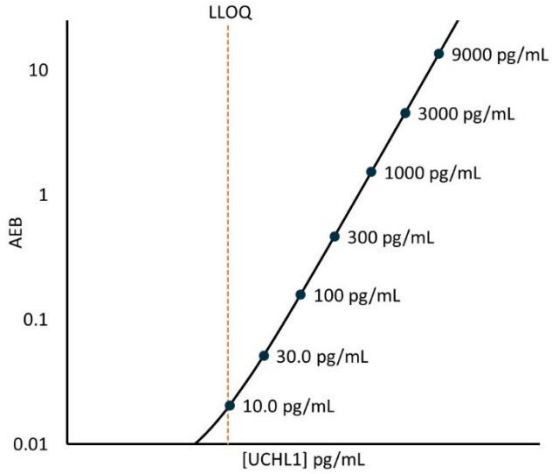
GFAP Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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.

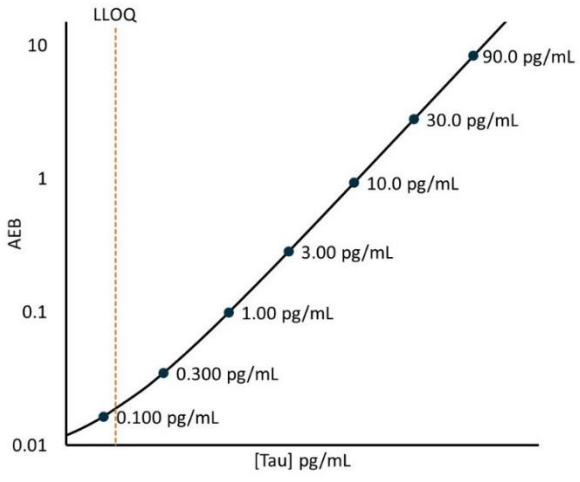
UCH-L1 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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.



Minimum Required Dilution (MRD)

Diluted Sample Volume	100 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 over 6 runs each for 1 reagent lot across 2 instruments (6 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	**0.500 pg/mL** pooled CV 12% mean recovery 99%	**2.00 pg/mL**
GFAP	**9.38 pg/mL** pooled CV 10% mean recovery 93%	**37.5 pg/mL**
UCH-L1	**9.38 pg/mL** pooled CV 20% mean recovery 115%	**37.5 pg/mL**
Tau	**0.125 pg/mL** pooled CV 19% mean recovery 99%	**0.500 pg/mL**

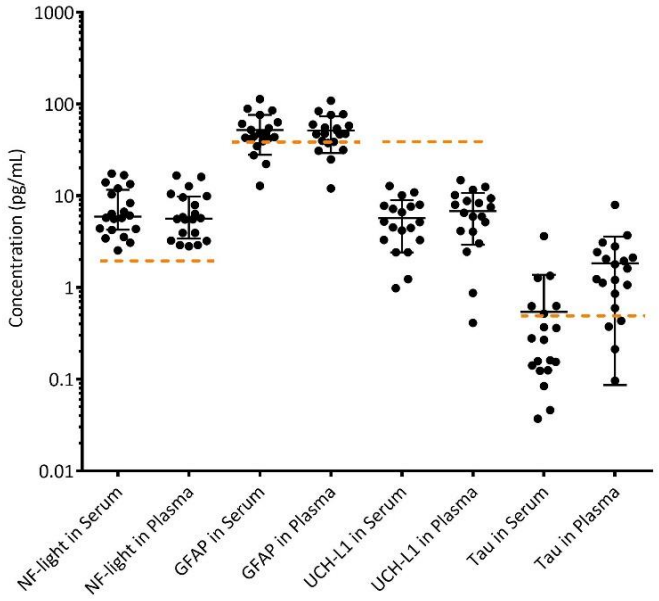
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 6 runs each for 1 reagent lot across 2 instruments (6 runs total).

	LOD
NF-light	**0.105 pg/mL** range 0.0423-0.166 pg/mL
GFAP	**1.51 pg/mL** range 0.335-3.22 pg/mL
UCH-L1	**1.90 pg/mL** range 0.855-3.17 pg/mL
Tau	**0.0408 pg/mL** range 0.0168-0.0908 pg/mL

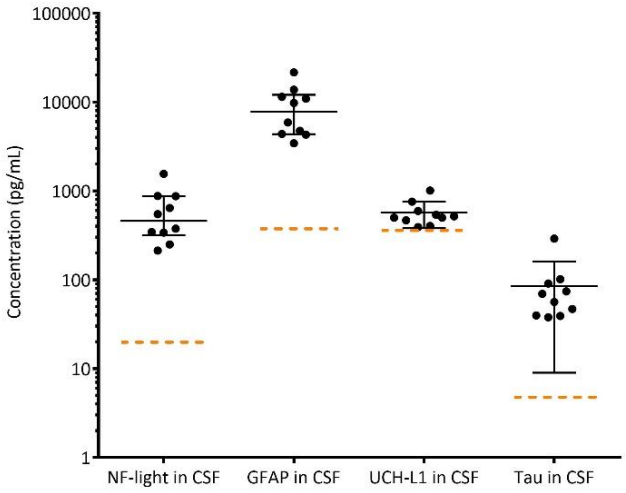
Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The ranges below are for serum and plasma. The Upper Limit of Quantification (ULOQ) for CSF is 10X the ULOQ for serum and plasma.

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

Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=20), and serum (n=20) were measured. One UCH-L1 serum sample, one UCH-L1 plasma sample, and one Tau serum sample could not be detected. Bars depict median with interquartile range. Orange line represents functional LLOQ.



Endogenous CSF Readings: CSF (n=10) 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	7.03	5.63	100%	100%
	Serum	7.69	5.91	100%	100%
	CSF	602	462	100%	100%
GFAP	Plasma	59.4	54.0	100%	75%
	Serum	58.9	50.0	100%	80%
	CSF	9027	7829	100%	100%
UCH-L1	Plasma	*	*	95%	0%
	Serum	*	*	95%	0%
	CSF	569	510	100%	100%
Tau	Plasma	2.22	1.87	100%	85%
	Serum	1.33	0.948	95%	30%
	CSF	84.6	62.8	100%	100%

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

*No values above LLOQ

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

NF-L	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	6.05	7.6%	10.2%	10.9%
Control 2	585	3.3%	7.1%	2.3%
Panel 1	3.99	8.3%	5.4%	1.0%
Panel 2	33.3	4.6%	8.2%	4.1%
Panel 3	405	6.3%	7.0%	6.8%

GFAP	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	90.9	13.4%	5.5%	5.6%
Control 2	10219	4.1%	9.2%	3.0%
Panel 1	45.2	10.5%	4.7%	1.9%
Panel 2	175	7.2%	7.3%	0.9%
Panel 3	2535	8.0%	6.5%	4.3%

UCH-L1	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	107	14.7%	7.9%	3.2%
Control 2	10435	3.3%	5.9%	0.1%
Panel 1	58.1	11.2%	7.3%	5.3%
Panel 2	326	5.6%	5.6%	0.8%
Panel 3	4806	5.4%	5.7%	0.2%

Tau	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	3.84	7.3%	7.1%	0.7%
Control 2	210	2.4%	3.8%	0.7%
Panel 1	1.82	8.2%	9.0%	7.9%
Panel 2	39.4	4.0%	6.9%	3.9%
Panel 3	182	4.3%	5.4%	4.9%

Spike and Recovery, Serum and Plasma: 7 serum and 7 EDTA plasma samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

	Recovery
NF-light	**78%** range 75-83%
GFAP	**53%** range 38-66%
UCH-L1	**52%** range 35-82%
Tau	**75%** range 48-108%

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

	Recovery
NF-light	**109%** range 108-109%
GFAP	**110%** range 103-117%
UCH-L1	**101%** range 99-103%
Tau	**112%** range 110-113%

Dilution Linearity, Serum: 5 spiked serum and 5 spiked EDTA plasma samples were diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

	Linearity
NF-light	**117%** range 47-139%
GFAP	**141%** range 63-187%
UCH-L1	**163%** range 79-195%
Tau	**130%** range 45-176%

Dilution Linearity, CSF: 2 spiked CSF samples were diluted 2x serially from MRD (40x) to 640x with Sample Diluent.

	Linearity
NF-light	**84%** range 81-87%
GFAP	**94%** range 93-94%
UCH-L1	**84%** range 82-85%
Tau	**93%** range 88-98%

The Simoa N4PB assay is formulated for use on the SR-X®, HD-1, or HD-X® platform. Some differences in performance claims between the 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 may be due to minor differences in antibody and analyte behavior in the different assay formats.