Simoa® N4PA Advantage Kit
HD-1/HD-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]

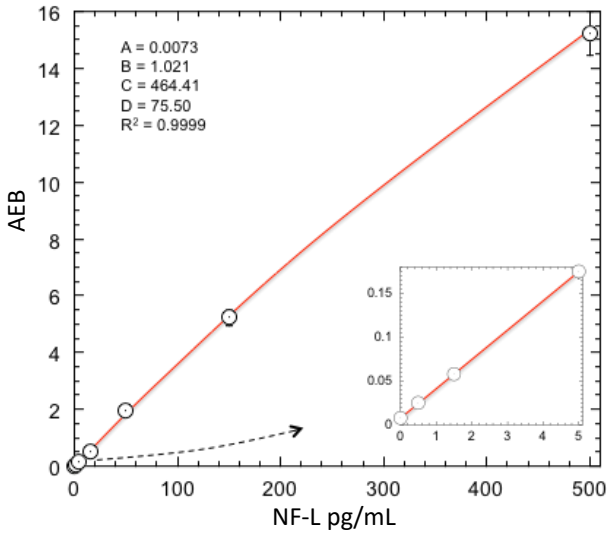
Description – NF-light Test

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.

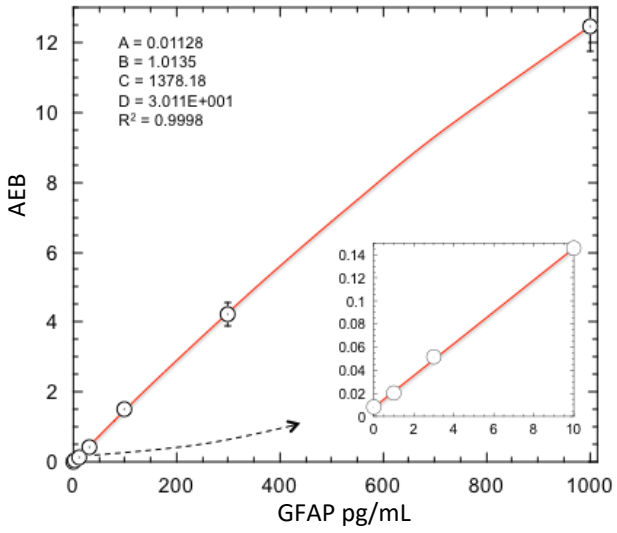
Description – GFAP Test

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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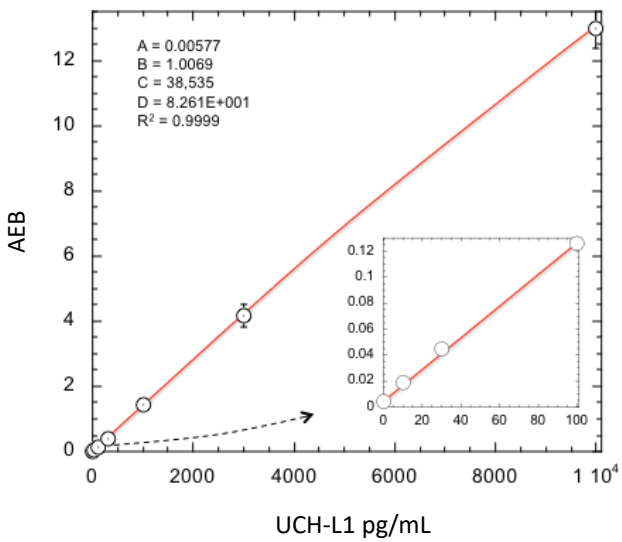
Description – UCH-L1 Test

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.

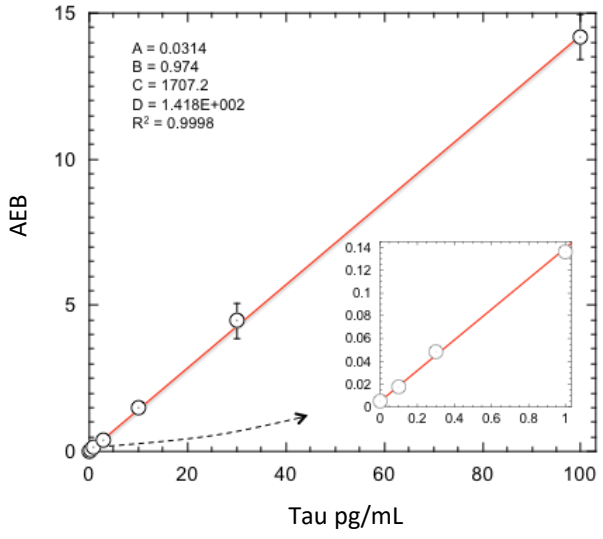
UCH-L1 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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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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).

NF-L LLOQ	**0.241 pg/mL** pooled CV 12.0% mean recovery 109.8%
GFAP LLOQ	**0.467 pg/mL** pooled CV 12.9% mean recovery 102.8%
UCH-L1 LLOQ	**5.45 pg/mL** pooled CV 15.0% mean recovery 108.9%
Tau LLOQ	**0.053 pg/mL** pooled CV 12.2% mean recovery 108.9%

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).

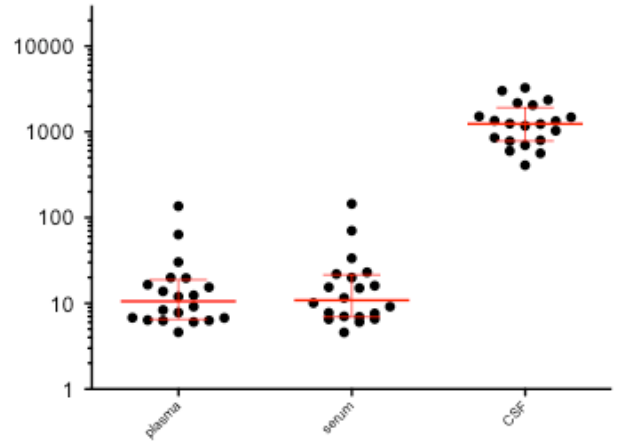
NF-L LOD	**0.104 pg/mL** range 0.025-0.276 pg/mL
GFAP LOD	**0.221 pg/mL** range 0.042-0.481 pg/mL
UCH-L1 LOD	**1.74 pg/mL** range 0.120-3.48 pg/mL
Tau LOD	**0.024 pg/mL** range 0.007-0.059 pg/mL

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

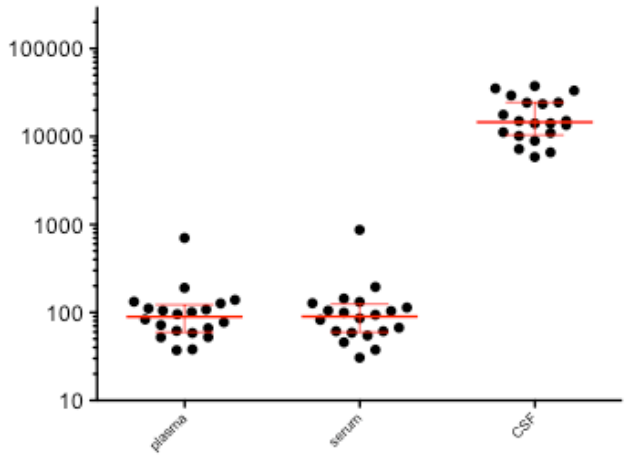
NF-L Dynamic range	0-2000 pg/mL
GFAP Dynamic range	0-4000 pg/mL
UCH-L1 Dynamic range	0-40 ng/mL
Tau Dynamic range	0-400 pg/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

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

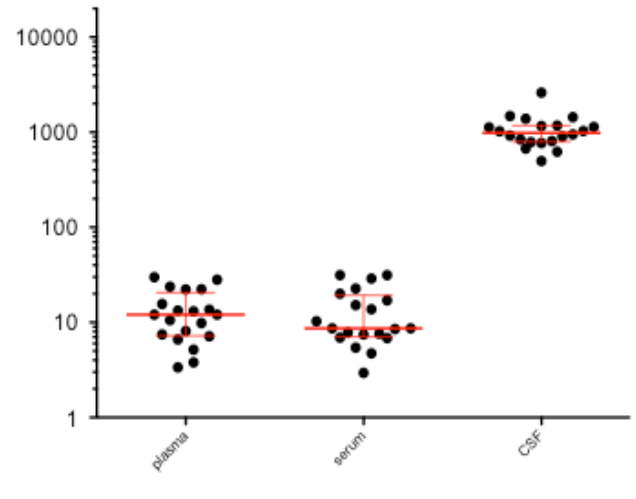
Endogenous Sample Readings (NF-L): NF-L in matched EDTA plasma and serum (n=20) and CSF (n=20) from healthy donors. Error bars depict mean and interquartile ranges.



Endogenous Sample Readings (GFAP): GFAP in matched EDTA plasma and serum (n=20) and CSF (n=20) from healthy donors. Error bars depict mean and interquartile ranges.

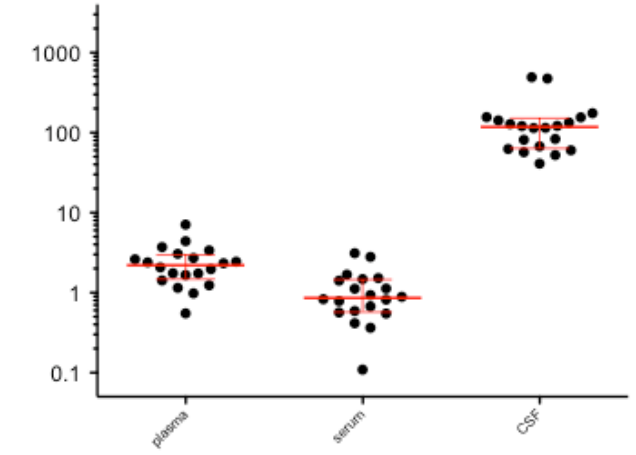


Endogenous Sample Readings (UCH-L1): UCH-L1 in matched EDTA plasma and serum (n=20) and CSF (n=20) from healthy donors. Error bars depict mean and interquartile ranges.

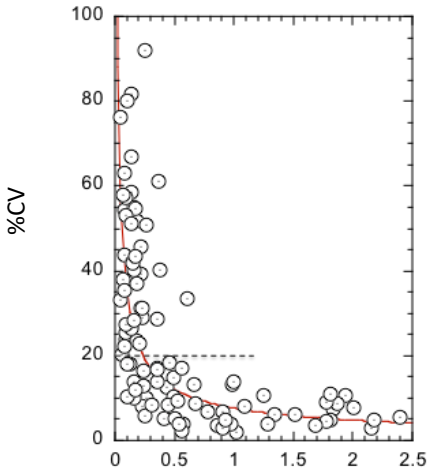


Target	Sample Type	Median Conc pg/mL	% Above LOD
NF-L	EDTA plasma	10.6	100%
	Serum	10.8	100%
	CSF	1241	100%
GFAP	EDTA plasma	89.7	100%
	Serum	90.2	100%
	CSF	14624	100%
UCH-L1	EDTA plasma	12.1	100%
	Serum	8.66	100%
	CSF	989	100%
Tau	EDTA plasma	2.21	100%
	Serum	0.861	100%
	CSF	118	100%

Endogenous Sample Readings (Tau): Tau in matched EDTA plasma and serum (n=20) and CSF (n=20) from healthy donors. Error bars depict mean and interquartile ranges.

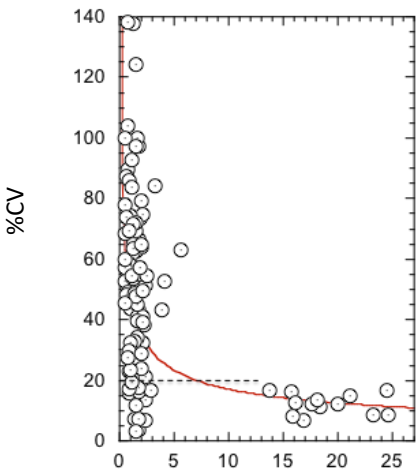


Dose CV Profile (NF-L): Diluted serum was assayed in reps of 3 over multiple days and runs (104 determinations).



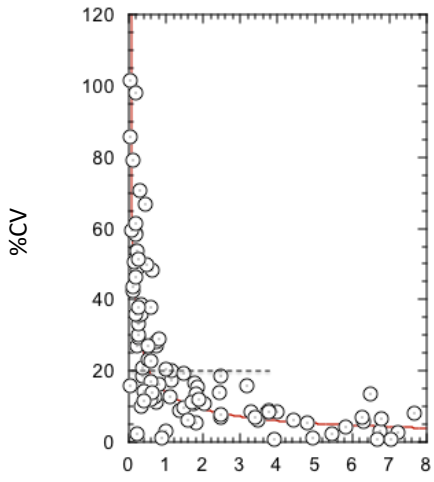
Mean NF-L pg/mL

Dose CV Profile (UCH-L1): Diluted serum was assayed in reps of 3 over multiple days and runs (104 determinations).



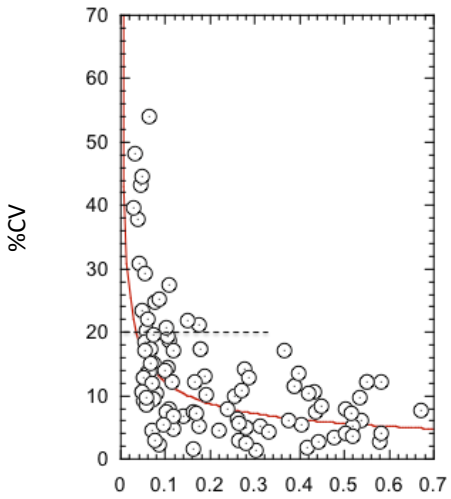
Mean UCH-L1 pg/mL

Dose CV Profile (GFAP): Diluted serum was assayed in reps of 3 over multiple days and runs (53 determinations).



Mean GFAP pg/mL

Dose CV Profile (Tau): Diluted serum was assayed in reps of 3 over multiple days and runs (104 determinations).



Mean Tau pg/mL

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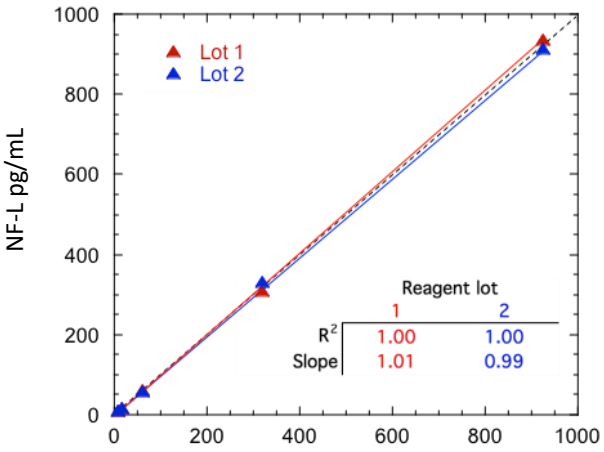
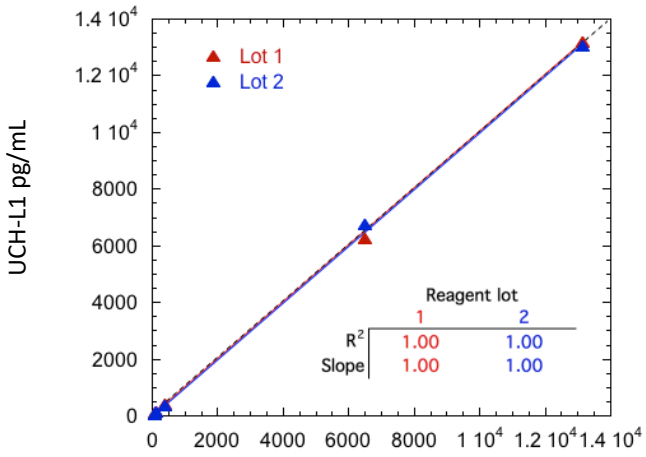
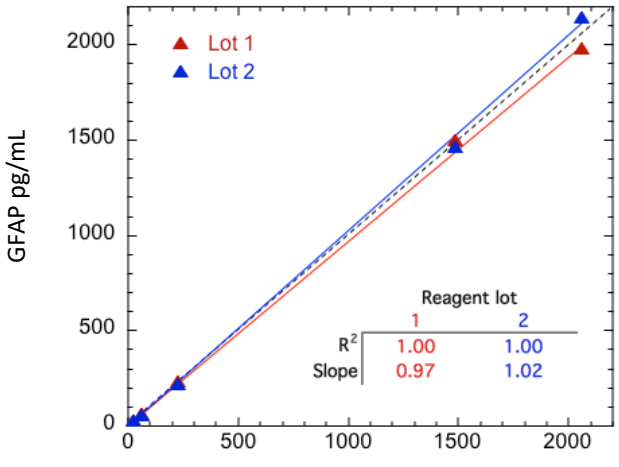
Reproducibility Across Reagent Lots (NF-L): Five native and spiked serum and plasma samples tested across 2 runs x 3 instruments each lot.



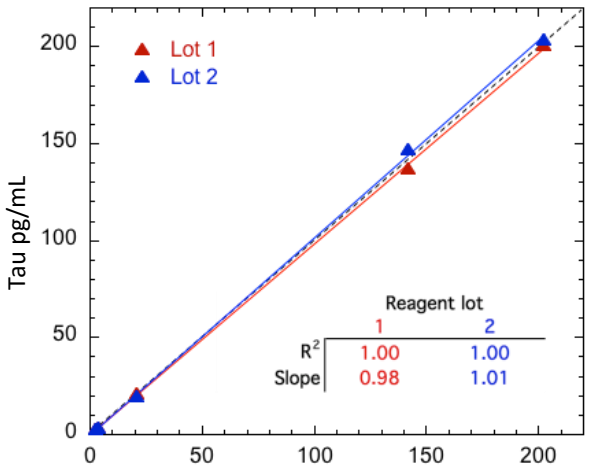
Reproducibility Across Reagent Lots (UCH-L1): Five native and spiked serum and plasma samples tested across 2 runs x 3 instruments each lot.



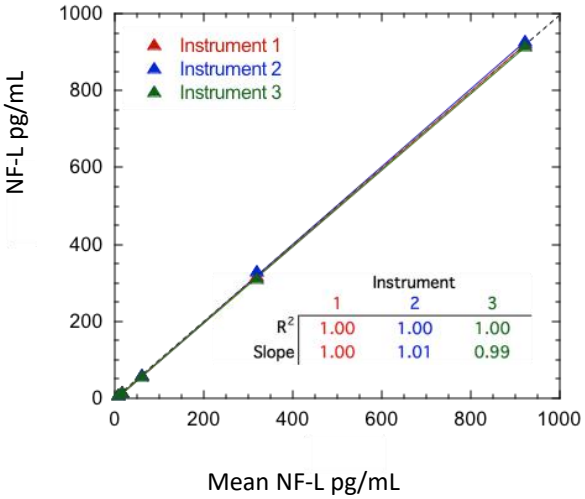
Reproducibility Across Reagent Lots (GFAP): Five native and spiked serum and plasma samples tested across 2 runs x 3 instruments each lot.



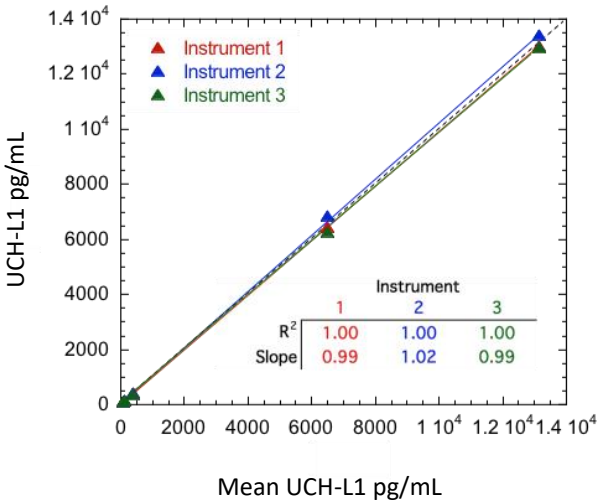
Reproducibility Across Reagent Lots (Tau): Five native and spiked serum and plasma samples tested across 2 runs x 3 instruments each lot.



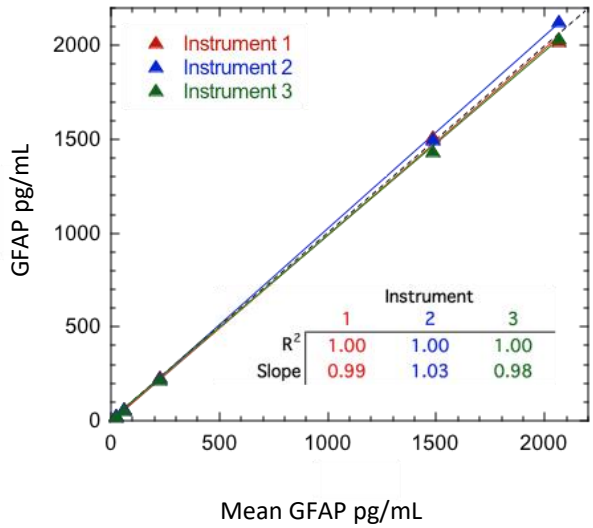
Reproducibility Across Instruments (NF-L): Five native and spiked serum and plasma samples tested across 2 runs x 2 reagent lots each instrument.



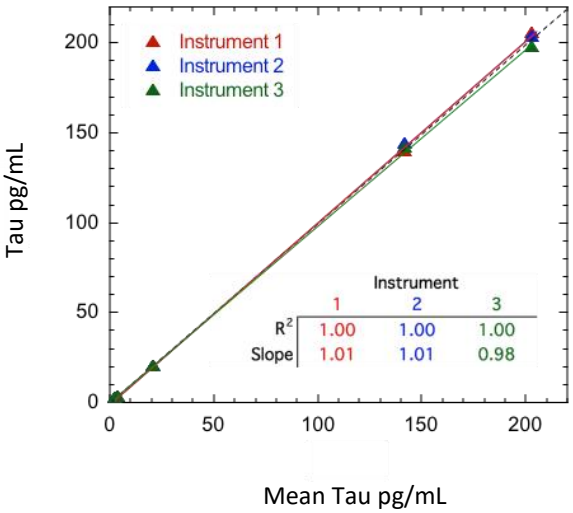
Reproducibility Across Instruments (UCH-L1): Five native and spiked serum and plasma samples tested across 2 runs x 2 reagent lots each instrument.



Reproducibility Across Instruments (GFAP): Five native and spiked serum and plasma samples tested across 2 runs x 2 reagent lots each instrument.



Reproducibility Across Instruments (Tau): Five native and spiked serum and plasma samples tested across 2 runs x 2 reagent lots each instrument.



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Reproducibility Precision (NF-L): Five samples consisting of two serum panels, one plasma panel, and two NF-L controls were assayed in replicates of three for two runs on each of three instruments and two reagent lots. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	16.8	0.0%	0.0%	5.1%	5.4%
Plasma Panel 2	60.1	0.0%	0.0%	5.6%	4.7%
Serum Panel 3	332	0.0%	3.7%	5.0%	4.7%

Reproducibility Precision (GFAP): Five samples consisting of two serum panels, one plasma panel, and two GFAP controls were assayed in replicates of three for two runs on each of three instruments and two reagent lots. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	59.4	0.0%	8.5%	3.8%	3.9%
Plasma Panel 2	226	0.0%	3.0%	6.2%	3.6%
Serum Panel 3	2095	0.0%	6.5%	3.8%	4.5%

Reproducibility Precision (UCH-L1): Five samples consisting of two serum panels, one plasma panel, and two UCH-L1 controls were assayed in replicates of three for two runs on each of three instruments and two reagent lots. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	71.9	5.0%	4.3%	6.4%	11.3%
Plasma Panel 2	381	4.8%	3.5%	4.9%	5.6%
Serum Panel 3	6663	0.0%	3.5%	5.9%	4.2%

Reproducibility Precision (Tau): Five samples consisting of two serum panels, one plasma panel, and two Tau controls were assayed in replicates of three for two runs on each of three instruments and two reagent lots. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	2.66	0.0%	0.0%	5.7%	6.7%
Plasma Panel 2	20.2	1.5%	0.0%	6.6%	4.9%
Serum Panel 3	148	5.2%	0.0%	3.0%	5.2%

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Repeatability Precision (NF-L): Five samples consisting of two serum panels, one plasma panel, and two NF-L controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	16.1	0.0%	4.4%	6.0%
Plasma Panel 2	61.5	0.0%	0.0%	4.6%
Serum Panel 3	325	0.0%	7.8%	4.5%

Repeatability Precision (UCH-L1): Five samples consisting of two serum panels, one plasma panel, and two UCH-L1 controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	73.0	0.0%	10.6%	9.6%
Plasma Panel 2	356	4.0%	6.7%	5.6%
Serum Panel 3	6082	0.0%	7.2%	4.2%

Repeatability Precision (GFAP): Five samples consisting of two serum panels, one plasma panel, and two GFAP controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	60.5	2.6%	2.0%	5.6%
Plasma Panel 2	221	0.0%	4.0%	4.2%
Serum Panel 3	1910	0.0%	8.5%	5.0%

Repeatability Precision (Tau): Five samples consisting of two serum panels, one plasma panel, and two Tau controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

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%
Serum Panel 1	2.66	0.0%	12.4%	7.4%
Plasma Panel 2	20.6	0.0%	7.6%	4.3%
Serum Panel 3	142	0.0%	6.2%	4.3%

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Inter Lot CV: Pool of CVs from 5 samples tested with 2 reagent lots across 2 runs x 3 instruments.

NF-L	**3.5%** Sample range: 9.28-320 pg/mL
GFAP	**4.4%** Sample range: 27.3-2062 pg/mL
UCH-L1	**3.9%** Sample range: 22.6-389 pg/mL
Tau	**3.0%** Sample range: 2.76-202.6 pg/mL

Inter Instrument CV: Pool of CVs from 5 samples tested with 3 instruments across 2 runs x 2 reagent lots.

NF-L	**2.5%** Sample range: 9.28-320 pg/mL
GFAP	**4.7%** Sample range: 27.3-2062 pg/mL
UCH-L1	**4.5%** Sample range: 22.6-389 pg/mL
Tau	**3.6%** Sample range: 2.76-202.6 pg/mL

Spike and Recovery, Serum and Plasma (NF-L): NF-L spiked into 2 serum, 2 plasma samples at 50 and 500 pg/mL.

Spike and Recovery, Serum and Plasma (GFAP): GFAP spiked into 2 serum, 2 plasma samples at 80 and 800 pg/mL.

Spike and Recovery, Serum and Plasma (UCH-L1): UCH-L1 spiked into 2 serum, 2 plasma samples at 300 and 3000 pg/mL.

Spike and Recovery, Serum and Plasma (Tau): Tau 441 spiked into 2 serum, 2 plasma samples at 15 and 150 pg/mL.

NF-L	**67.5%** Range 54.4-85.1%
GFAP	**77.2%** Range: 49.3-119.6%
UCH-L1	**106.9%** Range: 87.3-124.7%
Tau	**112.5%** Range: 76.7-157.9%

Spike and Recovery, CSF (NF-L): NF-L spiked into 4 CSF samples at 500 and 5000 pg/mL.

Spike and Recovery, CSF (GFAP): GFAP spiked into 4 CSF samples at 1,500 and 15,000 pg/mL.

Spike and Recovery, CSF (UCH-L1): UCH-L1 spiked into 4 CSF samples at 15,000 and 150,000 pg/mL.

Spike and Recovery, CSF (Tau): Tau 411 spiked into 4 CSF samples at 100 and 1000 pg/mL.

NF-L	**107.7%** Range 88.8-124.9%
GFAP	**118.9%** Range: 87.5-176.7%
UCH-L1	**118.9%** Range: 96.0-167.2%
Tau	**116.3%** Range: 96.3-137.8%

Admixture Linearity (NF-L): High NF-L plasma sample fractionally admixed with low NF-L serum sample, mean of 10 levels.

Admixture Linearity (GFAP): High GFAP serum sample fractionally admixed with low tau plasma sample, mean of 10 levels.

Admixture Linearity (UCH-L1): High UCH-L1 serum sample fractionally admixed with low tau serum sample, mean of 10 levels.

Admixture Linearity (Tau): High tau serum sample fractionally admixed with low tau plasma sample, mean of 10 levels.

NF-L	**Mean = 94.9%** Range: 87.6-101.3%
GFAP	**Mean = 100.6%** Range: 88.9-112.2%
UCH-L1	**Mean = 95.4%** Range: 85.1-104.0%
Tau	**Mean = 94.3%** Range: 83.4-105.6%

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

NF-L (128x)	**Mean = 118.8%** Range: 101.0-155.2%
GFAP (128x)	**Mean = 108.3%** Range: 102.3-120.9%
UCH-L1 (128x)	**Mean = 90.1%** Range: 79.0-103.0%
Tau (128x)	**Mean = 115.0%** Range: 106.1-125.5%

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

NF-L (1280x)	**Mean = 99.9%** Range: 92.6-109.9%
GFAP (1280x)	**Mean = 98.1%** Range: 95.5-103.6%
UCH-L1 (1280x)	**Mean = 95.8%** Range: 93.5-98.0%
Tau (1280x)	**Mean = 91.6%** Range: 78.1-121.2%

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.

References

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