Simoa® Neurology 4-Plex D Advantage PLUS Kit

HD-X Data Sheet Item 104822

Description: This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the N4PD Advantage PLUS kit on the HD-X platform. Data provided includes Calibration Curves, Minimum Required Dilution (MRD), Lower Limit of Qualification (LLOQ), Limit of Detection (LOD), Assay Range, Precision, Spike and Recovery, Dilution Linearity, and Cross-Reactivity.

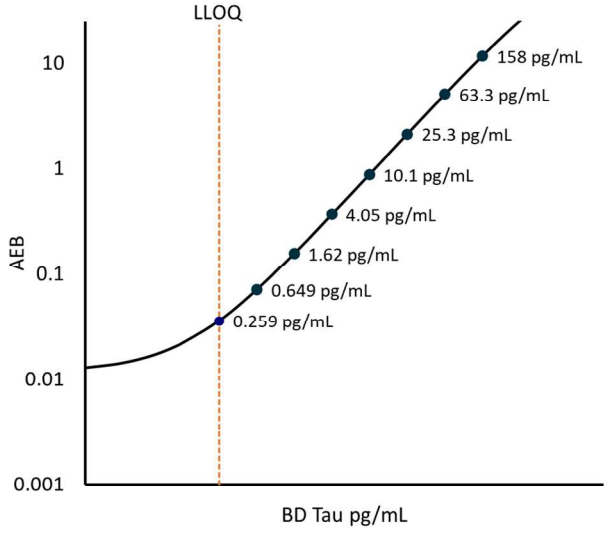
BD-Tau: Brian Derived (BD) Tau has been shown to be a more specific measurement of neurodegenerative disease than total Tau in blood in that it allows for discrimination of brain Tau from those originating from other tissues. Moreover, blood BD-Tau levels correlate with BD-Tau in CSF. BD-Tau is emerging as a blood biomarker that outperforms total Tau and NF-L in distinguishing Alzheimer's disease (AD) from other neurodegenerative diseases. Precise measurement of BD-Tau provides a valuable tool in characterizing the role of BD-Tau in AD and will prove valuable in both diagnostic and clinical trial settings.

NF-L: 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.

GFAP: 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 can form 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. As a potential biomarker, GFAP 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.

UCH-L1: 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.

Calibration Curves: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

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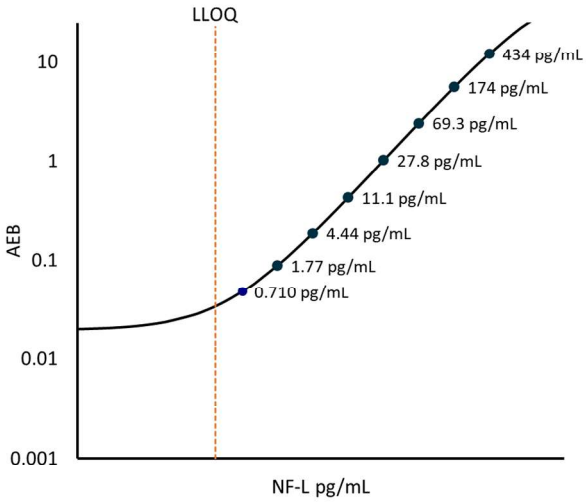

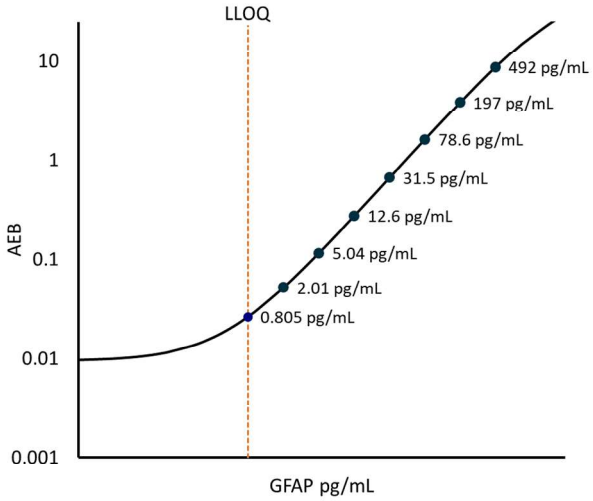

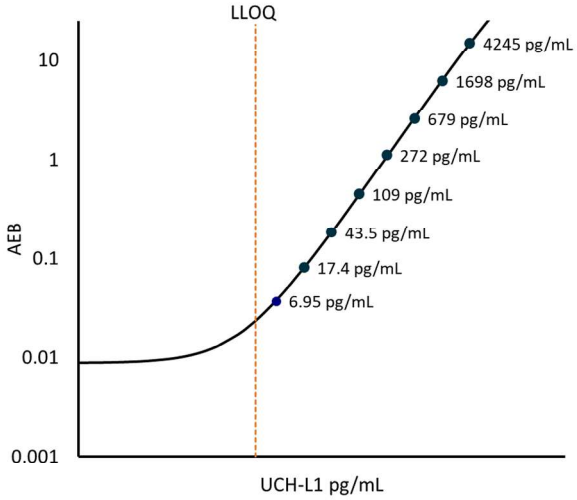

Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Serum and EDTA Plasma Dilution	1:4
CSF Dilution	1:100
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV < 20%. The functional LLOQ values below represent the analytical LLOQ multiplied by the dilution factor used for the samples.

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

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The representative ranges below are for serum and EDTA plasma. The Upper Limit of Quantification (ULOQ) for CSF is 25x the ULOQ for serum and EDTA plasma. Note that the top concentration will vary between kit lots, as calibrators are value assigned to maintain consistency of results across lots.

BD Tau	
Analytical LLOQ	0.259 pg/mL Pooled CV: 10.7% Mean Recovery: 99.6%
Functional LLOQ	Serum/EDTA Plasma (4x): 1.04 pg/mL CSF (100x): 25.9 pg/mL
Functional ULOQ	Serum/EDTA Plasma (4x): 600 pg/mL CSF (100x): 15 ng/mL
LOD	0.029 pg/mL Range: 0.002 - 0.049 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 600 pg/mL CSF (100x): 0 - 15 ng/mL

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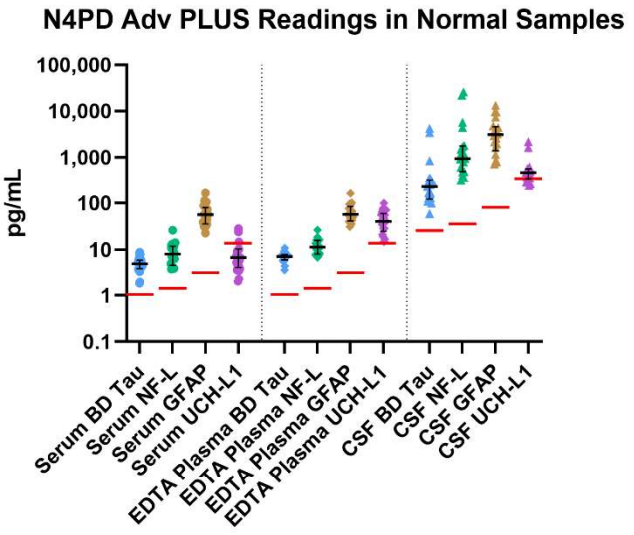
NF-L	
Analytical LLOQ	**0.355 pg/mL** Pooled CV: 19.6% Mean Recovery: 92.9%
Functional LLOQ	Serum/EDTA Plasma (4x): 1.42 pg/mL CSF (100x): 35.5 pg/mL
Functional ULOQ	Serum/EDTA Plasma (4x): 1800 pg/mL CSF: 45 ng/mL
LOD	**0.094 pg/mL** Range: 0.021 - 0.184 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 1800 pg/mL CSF (100x): 0 - 45 ng/mL

GFAP	
Analytical LLOQ	**0.805 pg/mL** Pooled CV: 11.1% Mean Recovery: 100.7%
Functional LLOQ	Serum/EDTA Plasma (4x): 3.22 pg/mL CSF (100x): 80.5 pg/mL
Functional ULOQ	Serum/EDTA Plasma (4x): 3200 pg/mL CSF (100x): 80 ng/mL
LOD	**0.121 pg/mL** Range: 0.029 - 0.282 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 3200 pg/mL CSF (100x): 0 - 80 ng/mL

UCH-L1	
Analytical LLOQ	**3.48 pg/mL** Pooled CV: 13.9% Recovery: 91.6%
Functional LLOQ	Serum/EDTA Plasma (4x): 13.9 pg/mL CSF (100x): 348 pg/mL
Functional ULOQ	Serum/EDTA Plasma (4x): 16000 pg/mL CSF: 400 ng/mL
LOD	**0.577 pg/mL** Range: 0.204 - 2.04 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 16000 pg/mL CSF (100x): 0 - 400 ng/mL

Endogenous Sample Reading: Concentrations (pg/mL) were determined for unmatched CSF (n=20) and matched serum (n=20) and EDTA plasma (n=20) from normal human donors using the N4PD Advantage PLUS Assay kit on HD-X. Bars depict median with interquartile range. The red lines represent functional LLOQ.



BD Tau				
Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
Serum	5.14	5.05	100%	100%
EDTA Plasma	7.22	7.40	100%	100%
CSF	597	236	100%	100%

NF-L				
Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
Serum	9.47	8.46	100%	100%
EDTA Plasma	12.6	11.4	100%	100%
CSF	3631	1011	100%	100%

GFAP				
Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
Serum	63.9	57.0	100%	100%
EDTA Plasma	65.4	58.5	100%	100%
CSF	4072	3161	100%	100%

UCH-L1				
Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
Serum	N/A*	N/A*	95%	15%
EDTA Plasma	46.2	43.4	100%	100%
CSF	675**	504**	100%	75%

* Mean and Median are not included because of low sample quantifiability.
** Values below LLOQ are excluded from the mean and median calculation.

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Precision: Measurements of 2 serum-based panels, 2 EDTA plasma-based panels, 2 CSF-based panels, and 2 calibrator-based controls. Triplicate measurements were made for 12 runs each for across 2 instruments (12 runs total, 48 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

BD Tau					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	8.08	4.5%	12.6%	4.2%	2.0%
Control 2	318	2.7%	8.4%	3.7%	3.7%
Panel 1	14.9	3.3%	11.2%	0.7%	0.1%
Panel 2	133	3.9%	10.3%	4.5%	2.9%
Panel 3	27.0	3.4%	9.6%	3.1%	1.2%
Panel 4	134	4.7%	10.6%	4.3%	6.9%
Panel 5	5077	3.1%	9.2%	1.6%	7.1%
Panel 6	14012	3.7%	11.6%	1.7%	6.8%

NF-L					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	23.6	3.7%	15.2%	7.5%	5.2%
Control 2	953	2.7%	11.9%	9.2%	4.8%
Panel 1	52.9	3.7%	16.5%	11.4%	3.1%
Panel 2	252	4.0%	11.8%	9.1%	3.5%
Panel 3	61.3	3.6%	15.2%	10.9%	6.6%
Panel 4	290	4.3%	14.7%	10.1%	9.8%
Panel 5	39278	4.3%	11.2%	4.5%	8.1%
Panel 6	41082	5.3%	15.7%	5.4%	8.1%

GFAP					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	24.8	4.0%	16.7%	1.7%	1.9%
Control 2	960	2.9%	8.1%	3.4%	0.9%
Panel 1	98.8	3.7%	16.8%	9.2%	0.2%
Panel 2	490	6.4%	10.8%	1.3%	4.6%
Panel 3	68.3	4.7%	11.4%	4.9%	0.9%
Panel 4	484	4.8%	9.8%	4.3%	4.8%
Panel 5	9803	3.4%	15.7%	0.7%	8.0%
Panel 6	59395	4.3%	14.8%	1.8%	5.4%

UCH-L1					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	162	4.1%	14.5%	3.9%	1.1%
Control 2	6399	2.6%	8.8%	4.1%	0.0%
Panel 1	245	3.5%	18.7%	4.9%	0.2%
Panel 2	2326	3.5%	11.8%	4.4%	3.5%
Panel 3	348	3.8%	11.7%	1.1%	1.5%
Panel 4	2258	3.5%	10.6%	3.5%	0.3%
Panel 5	30937	3.1%	9.8%	4.9%	0.5%
Panel 6	185934	2.9%	10.8%	5.1%	0.5%

Spike and Recovery: 4 serum, 4 EDTA plasma and 4 CSF samples were spiked at low and high concentrations within the range of the assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration in the spiked sample and the measured concentration in unspiked sample relative to the concentration in spiked plasma or CSF sample diluent, respectively. Results indicate that matrix effects are observed with this assay, as a limited dilution was chosen to maximize the detectability/quantifiability of the analyte in samples from healthy donors.

Dilution Linearity: 4 serum, 4 EDTA plasma, and 4 CSF samples were serially diluted 2x with sample diluent and then tested at MRD. Total dilution of each sample ranged from 4x to 256x. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

BD Tau	
Mean Spike and Recovery Serum	**100.7%** Range: 88.3 - 123.9%
Mean Spike and Recovery EDTA Plasma	**94.6%** Range: 78.3 - 107.9%
Mean Spike and Recovery CSF	**101.4%** Range: 99.4 - 103.9%
Mean Dilution Linearity Serum	**108.9%** Range: 98 - 124.6%
Mean Dilution Linearity EDTA Plasma	**104.6%** Range: 96.2 - 109.6%
Mean Dilution Linearity CSF	**105.9%** Range: 104.7 - 106.9%

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NF-L	
Mean Spike and Recovery Serum	**93.9%** Range: 87.7 - 105.1%
Mean Spike and Recovery EDTA Plasma	**88.7%** Range: 77.2 - 94.3%
Mean Spike and Recovery CSF	**92.2%** Range: 91.3 - 92.8%
Mean Dilution Linearity Serum	**108.3%** Range: 101.8 - 118.4%
Mean Dilution Linearity EDTA Plasma	**103.2%** Range: 97.3 - 111.8%
Mean Dilution Linearity CSF	**108.5%** Range: 106.2 - 110.9%

GFAP	
Mean Spike and Recovery Serum	**89.6%** Range: 80.1 - 106.8%
Mean Spike and Recovery EDTA Plasma	**86.3%** Range: 76.3 - 93.2%
Mean Spike and Recovery CSF	**101.8%** Range: 99 - 106.6%
Mean Dilution Linearity Serum	**122.4%** Range: 112.1 - 136.7%
Mean Dilution Linearity EDTA Plasma	**120.6%** Range: 109 - 135%
Mean Dilution Linearity CSF	**105.6%** Range: 102.3 - 109.5%

UCH-L1	
Mean Spike and Recovery Serum	**88.8%** Range: 82.5 - 106.9%
Mean Spike and Recovery EDTA Plasma	**86.6%** Range: 69.2 - 97.3%
Mean Spike and Recovery CSF	**107%** Range: 104.9 - 110.7%
Mean Dilution Linearity Serum	**116.8%** Range: 105.3 - 126.4%
Mean Dilution Linearity EDTA Plasma	**112.8%** Range: 102.6 - 121.5%
Mean Dilution Linearity CSF	**108.6%** Range: 104.2 – 114.0%

Cross-Reactivity: 6 Panels were tested with the N4PD detector and detector of each single analyte only. The detector cross-reactivity is determined by the percentage of each non-corresponding detector measurement of the analyte compared to the default detector. The same 6 panels were spiked with each one of the four calibrators then measured. The analyte cross-reactivity is determined as the spillover of each calibrator to the other analytes.

BD Tau		
Effector Analyte	Detector Cross-Reactivity	Analyte Cross-Reactivity
NF-L	0.13%	0.13%
GFAP	0.06%	0.13%
UCH-L1	0.09%	0.22%

NF-L		
Effector Analyte	Detector Cross-Reactivity	Analyte Cross-Reactivity
BD-Tau	0.%	0.51%
GFAP	0.01%	0.31%
UCH-L1	0.03%	0.76%

GFAP		
Effector Analyte	Detector Cross-Reactivity	Analyte Cross-Reactivity
BD-Tau	0.01%	0.45%
NF-L	0.13%	0.29%
UCH-L1	0.04%	0.62%

UCH-L1		
Effector Analyte	Detector Cross-Reactivity	Analyte Cross-Reactivity
BD-Tau	0.11%	0.14%
NF-L	0.20%	0.11%
GFAP	0.32%	0.08%

The Simoa N4PD Advantage PLUS Assay kit is formulated for use on the HD-X platform. Verification and validation results for the fully automated HD-X instrument are summarized here. Implementing this assay on the SR-X instrument may result in performance differences due to the manual steps involved in reagent preparation incubations, wash steps, and bead loading. Assay protocol may have to be modified to obtain equivalent results.

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