Simoa® Neurology 4-Plex E Advantage PLUS Kit
HD-X Data Sheet

Item 104465

HUMAN NEUROLOGY 4-PLEX E ADVANTAGE PLUS ASSAY (N4PE+)

The Simoa Human Neurology 4-Plex E (N4PE+) Advantage Plus Assay simultaneously measures four biomarkers in EDTA plasma and CSF. The targets are Abeta 40 (Aβ40), Abeta 42 (Aβ42), Glial Fibrillary Acidic Protein (GFAP), and Neurofilament light (NF-L). Each biomarker has been demonstrated to have significance for the investigation of neurodegeneration and brain injury, and to have utility for monitoring effectiveness of drug discovery and development for neurological disease. With this Neuro 4-Plex E Plus kit, we introduce novel Abeta 40 and Abeta 42 assays developed for highly specific and sensitive measurement of the concentrations of full length Abeta1-42 and Abeta1-40. This improved specificity for full-length Abeta1- 42 and Abeta1-40 is expected to be increasingly important for monitoring the effect of treatments that target specific Abeta isoforms and for target engagement in future clinical trials.

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the **Neurology 4-Plex E (N4PE) Advantage PLUS** kit on the HD-X platform.

Description– Aβ40 Test

Aβ40 is a 40 amino acid proteolytic product from the amyloid precursor protein (APP) that has gained attention as a biomarker correlating with Alzheimer disease (AD) onset, mild cognitive impairment, vascular dementia, and other cognitive disorders. Beta-secretase cleavage of APP initially results in the production of an APP fragment that is further cleaved by gamma-secretase at residues 40-42 to generate two main forms of amyloid beta, Aβ40 and Aβ42. Amyloid beta (Aβ) peptides (including a shorter Aβ38 isoform) are produced by different cell types in the body, but the expression is particularly high in the brain. Accumulation of Aβ in the form of extracellular plaques is a neuropathological hallmark of AD and believed to play a central role in the neurodegenerative process. Aβ40 is the major amyloid component in these plaques and is thought to be an initiating factor of AD plaques. In healthy and disease states Aβ40 is the most abundant form of the amyloid peptides in both cerebrospinal fluid (CSF) and plasma (10–20X higher than Aβ42). Recent studies suggest that a decrease in the ratio of Aβ42/Aβ40 may indicate AD progression. Determinations in serum samples are not reported due to high variability of Aβ40 in some healthy donor sample sets.

Description– Aβ42 Test

Aβ42 is a 42 amino acid proteolytic product from the amyloid precursor protein that has gained considerable attention as a biomarker correlating with Alzheimer disease (AD) onset, mild cognitive impairment, vascular dementia, and other cognitive disorders. Amyloid beta (Aβ) peptides (including the shorter Aβ38 and Aβ40 isoforms) are produced by many cell types in the body but the expression is particularly high in the brain. Accumulation of Aβ in the form of extracellular plaques is a neuropathological hallmark of AD and thought to play a central role in the neurodegenerative process. Substantial clinical validation has now been developed around disease relevance of cerebrospinal fluid (CSF) levels of Aβ42, and there follows a significant interest in measuring blood levels of this marker. Concentrations of Aβ42 in blood are over 100-fold lower than in cerebrospinal fluid, (typically single pg/mL range), requiring very high analytical sensitivity for its reliable measurement. Determinations in serum samples are not reported due to high variability of Aβ42 in some healthy donor sample sets.

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 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.

Description – NF-L 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 antibodies (Uman Diagnostics, Umeå Sweden) also cross react

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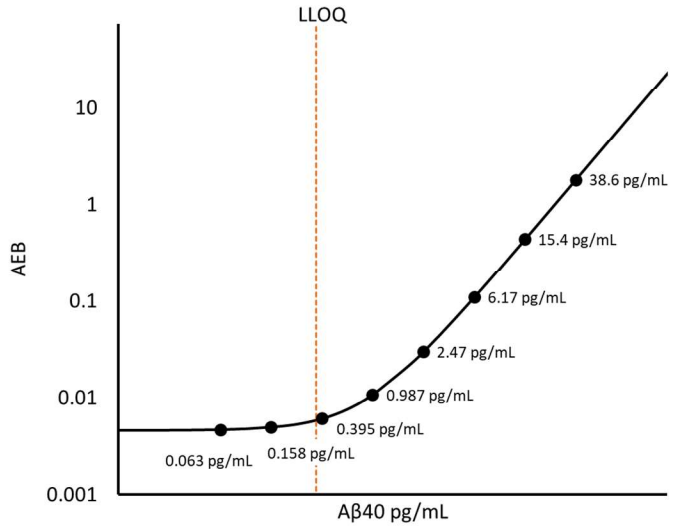
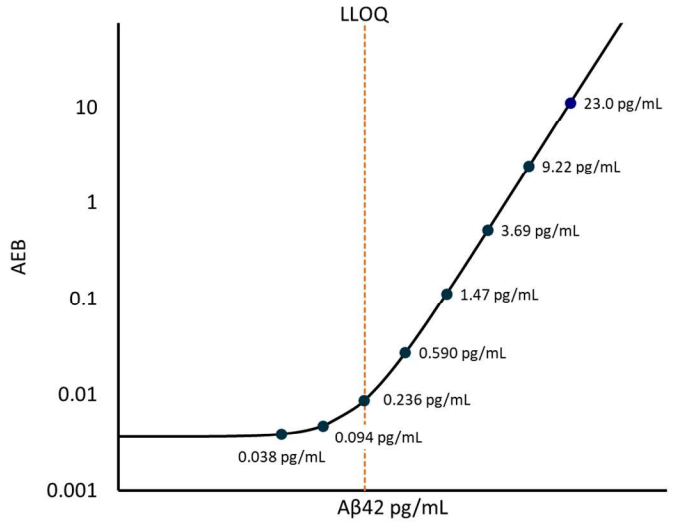
with murine, bovine, and macaque NF-L epitopes, and the assay can be used for research with these species.

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

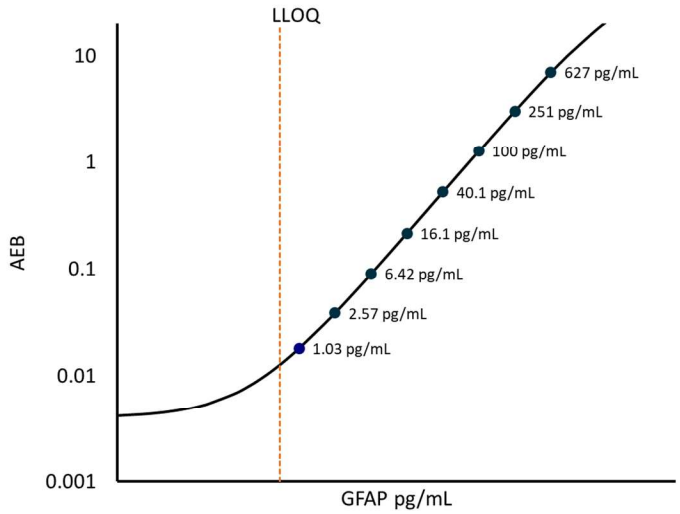
Aβ40 Curve: Calibrator concentrations and Lower Limit of Quantification.



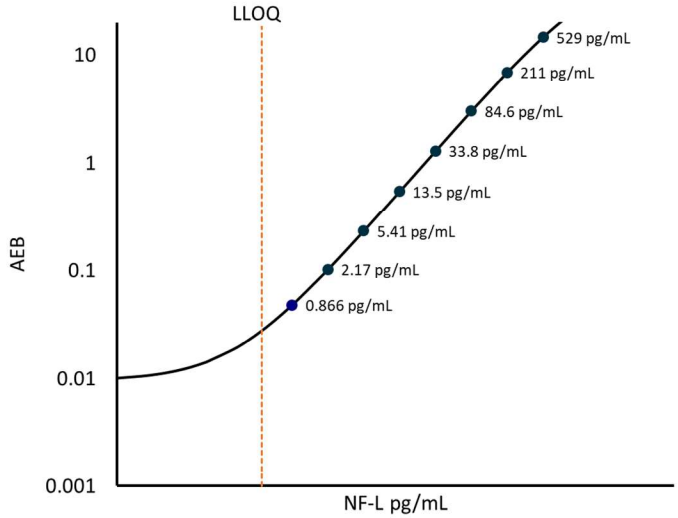
Aβ42 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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



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



Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Plasma Dilution	1:4
CSF Dilution	1:400
Tests per kit	96

See Kit Instruction for details.

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Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument). 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 (fLLOQ) values for each matrix represent the analytical LLOQ multiplied by the dilution factor used for the respective matrix (Table).

	Analytical LLOQ	Functional LLOQ	
		Plasma	CSF
Aβ40	0.353 pg/mL pooled CV 9.4% mean recovery 115%	1.41 pg/mL	141 pg/ml
Aβ42	0.239 pg/mL pooled CV 15.1% mean recovery 98.0%	0.957 pg/mL	95.7 pg/ml
GFAP	0.635 pg/mL pooled CV 15.5% mean recovery 97.6%	2.54 pg/mL	254 pg/ml
NF-L	0.402 pg/mL pooled CV 15.3% mean recovery 97.7%	1.61 pg/mL	161 pg/ml

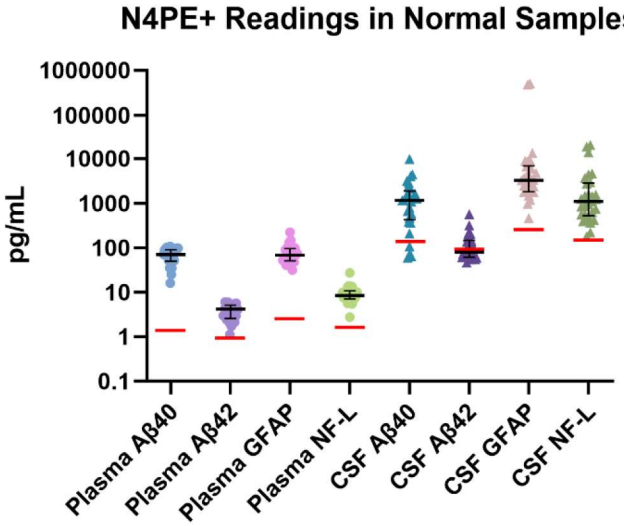
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument).

	LOD
Aβ40	**0.133 pg/mL** range 0.053 - 0.296 pg/mL
Aβ42	**0.130 pg/mL** range 0.046 - 0.244 pg/mL
GFAP	**0.139 pg/mL** range 0.031 - 0.342 pg/mL
NF-L	**0.072 pg/mL** range 0.009 - 0.156 pg/mL

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 EDTA plasma and CSF. Note that the top concentration will vary between kit lots to maintain consistency of results across lots.

	Assay Range	
	Plasma	CSF
Aβ40	0 - 180 pg/mL	0 - 18 ng/ml
Aβ42	0 - 80 pg/mL	0 - 8 ng/ml
GFAP	0 - 3200 pg/mL	0 - 320 ng/ml
NF-L	0 - 1800 pg/mL	0 - 180 ng/ml

Endogenous Sample Reading: Concentrations (pg/mL) were determined for EDTA plasma (*n*=26) and CSF (*n*=29) from normal human donors using the N4PE Advantage PLUS kit on HD-X. Bars depict median with interquartile range. The red lines represent functional LLOQ.



	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD	% Above LLOQ
Aβ40	Plasma	69.4	70.6	100	100
	CSF	1838	1270	94.8	86.2
Aβ42	Plasma	3.80	4.16	100	100
	CSF	197	158	86.2	46.6
GFAP	Plasma	80.4	68.5	100	100
	CSF	37858	3239	100	100
NF-L	Plasma	9.40	8.31	100	100
	CSF	3051	1090	100	100

Precision: 2 plasma-based panels, 2 CSF-based panels and 2 calibrator-based controls were measured for precision. Triplicate measurements were made for 6 runs each for 2 reagent lots across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

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Aβ40	Mean (pg/mL)	Within run CV	Between run CV	Between Inst CV	Between Lot CV
Control 1	3.64	4.2%	9.1%	0.0%	0.9%
Control 2	62.9	2.0%	5.5%	3.1%	0.8%
Panel 1	12.2	1.8%	5.9%	1.0%	0.0%
Panel 2	44.5	1.6%	4.2%	2.1%	2.1%
Panel 3	4494	1.9%	7.0%	1.9%	2.1%
Panel 4	8243	1.5%	10.6%	2.1%	2.2%

Aβ42	Mean (pg/mL)	Within run CV	Between run CV	Between Inst CV	Between Lot CV
Control 1	2.35	3.2%	11.9%	0.1%	1.8%
Control 2	36.1	1.8%	5.2%	0.6%	0.1%
Panel 1	8.32	2.3%	5.5%	0.4%	3.8%
Panel 2	26.6	1.3%	5.0%	2.3%	1.3%
Panel 3	329	4.1%	7.6%	0.4%	0.2%
Panel 4	463	2.8%	13.1%	0.9%	0.6%

GFAP	Mean (pg/mL)	Within run CV	Between run CV	Between Inst CV	Between Lot CV
Control 1	119	3.2%	10.5%	0.3%	1.4%
Control 2	1531	3.8%	6.3%	3.0%	1.5%
Panel 1	53.8	2.8%	9.5%	2.7%	0.6%
Panel 2	1184	3.3%	8.9%	3.8%	2.1%
Panel 3	7044	2.7%	12.7%	4.7%	2.1%
Panel 4	4053	3.1%	14.6%	0.5%	2.2%

NF-L	Mean (pg/mL)	Within run CV	Between run CV	Between Inst CV	Between Lot CV
Control 1	81.8	2.6%	9.8%	1.0%	1.9%
Control 2	1079	3.7%	7.2%	3.4%	0.0%
Panel 1	51.3	2.7%	7.0%	0.0%	3.0%
Panel 2	641	2.9%	8.1%	3.5%	1.6%
Panel 3	19964	3.9%	8.8%	1.2%	2.2%
Panel 4	1441	6.3%	10.3%	1.1%	3.9%

Spike Recovery: Two normal samples for each matrix (EDTA plasma and CSF) were spiked at low and high concentrations within the range of the assay and 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 in EDTA plasma with this assay, as a limited dilution was chosen
to maximize the detectability/quantifiability of the analyte in samples from healthy donors.

		Plasma	CSF
Aβ40	Spiked in concentration (pg/ml)	27.6 or 138	2759 or 13793
	Recovery %	83.7	111
	Range %	83.5 – 84.0	107 - 114
Aβ42	Spiked in concentration (pg/ml)	18.7 or 93.5	1869 or 9346
	Recovery %	78.4	106
	Range %	77.7 – 79.2	104 - 109
GFAP	Spiked in concentration (pg/ml)	524 or 2617	52394 or 261690
	Recovery %	71.7	106
	Range %	71.6 – 71.7	105 - 108
NF-L	Spiked in concentration (pg/ml)	116 or 576	11559 or 57579
	Recovery %	96.0	105
	Range %	93.2 – 98.8	103 - 107

Dilution Linearity: Two human samples for each matrix (EDTA plasma and CSF) were serially diluted with corresponding sample diluent through levels described in the table below. Each dilution series was run on the HD-X with two different lots of N4PE Advantage PLUS assay kits with the corresponding MRD (4X or 400X) dilution applied. Endogenous samples will be performed based on the sample availability.

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	Dilution Linearity			
	Matrix	Source	Dilution Factor	Result
Aβ40	Plasma	Endogenous	2X – 32X	85.6%
	Plasma	Endogenous	2X – 32X	99.8%
	CSF	spiked	2X – 64X	87.6%
	CSF	spiked	2X – 64X	87.9%
Aβ42	Plasma	spiked	2X – 32X	101%
	Plasma	spiked	2X – 32X	105%
	CSF	spiked	2X – 64X	104%
	CSF	spiked	2X – 64X	102%
GFAP	Plasma	Endogenous	2X – 16X	105%
	Plasma	Endogenous	2X – 16X	107%
	CSF	Endogenous	2X – 128X	94.1%
	CSF	Endogenous	2X – 128X	95.5%
NF-L	Plasma	spiked	2X – 128X	94.6%
	Plasma	spiked	2X – 128X	91.1%
	CSF	spiked	2X – 128X	94.3%
	CSF	spiked	2X – 128X	93.0%

The Simoa N4PE Advantage PLUS assay kit was designed and optimized for the fully automated HD-X platform. This report summarizes the verification and validation results for kit performance on the HD-X instrument. The semi-automated SR-X instrument employs a different workflow from HD-X, including some manual steps. Therefore, performance of this kit on the SR-X instrument may or may not match that of the HD-X. If assay performance on the SR-X does not match that of the HD-X, the assay protocol may be modified towards optimization on the SR-X platform.

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