Quanterix
The Science of Precision Health

Simoa™ N3PA Advantage Kit
SR-X Data Sheet

Item 101995

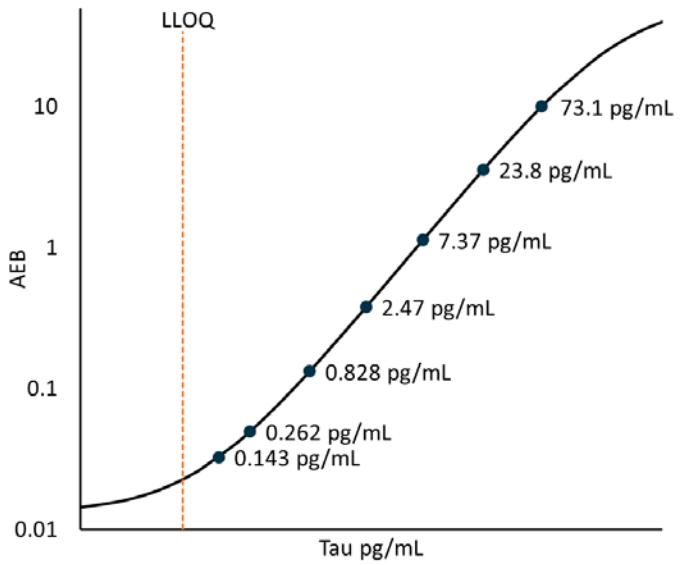
Description – Human Neurology 3-Plex "A"

The Simoa Human N3PA assay is a digital immunoassay for the quantitative determination of total Tau, Aβ42, and Aβ40 in human plasma and CSF. Determination in serum samples is not reported due to high variability of Aβ40 and Aβ42 in some healthy donor sample sets. This assay is for research use only and not for use in diagnostic procedures.

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 3-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.



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 high analytical sensitivity for its reliable measurement.

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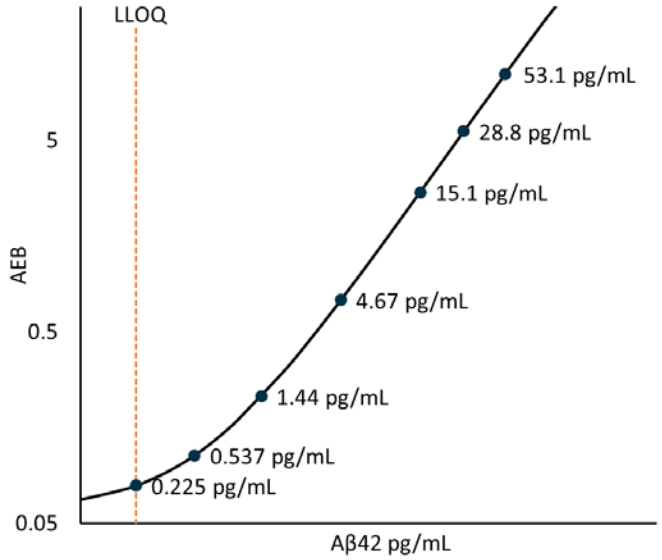
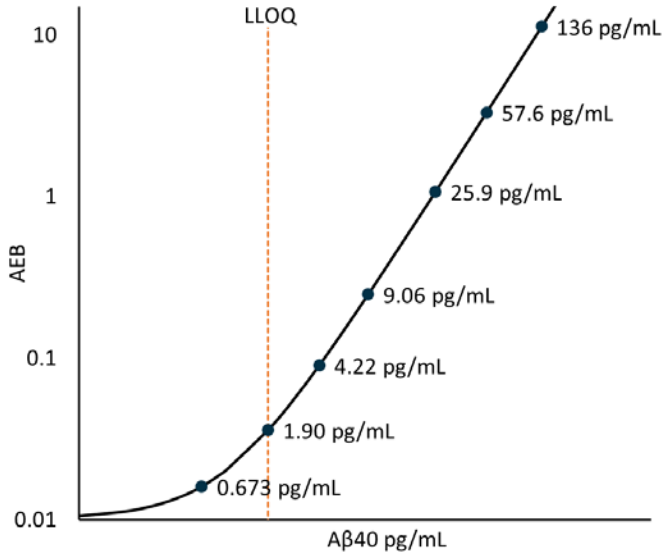
Aβ42 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Aβ40 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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 Aβ40/Aβ42 may indicate AD progression.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

Tau LLOQ	**0.0715 pg/mL** pooled CV 18% mean recovery 97%
Aβ42 LLOQ	**0.225 pg/mL** pooled CV 6% mean recovery 89%
Aβ40 LLOQ	**1.90 pg/mL** pooled CV 6% mean recovery 105%

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

Tau LOD	**0.0165 pg/mL** range 0.0141-0.0194 pg/mL
Aβ42 LOD	**0.147 pg/mL** range 0.0714-0.201 pg/mL
Aβ40 LOD	**0.243 pg/mL** range 0.0550-0.490 pg/mL

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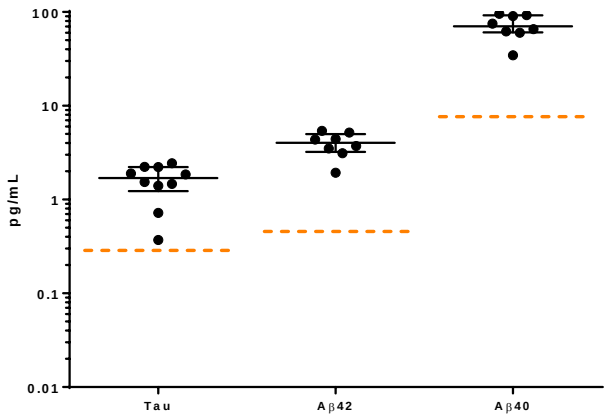
Sample Testing: The ranges listed below are for plasma. The ULOQ for CSF is 20X the ULOQ for plasma.

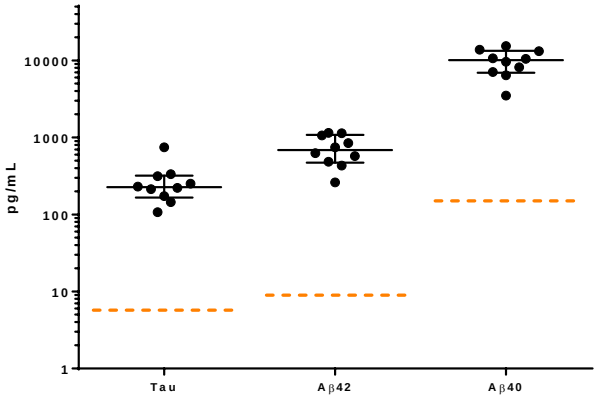
Tau Dynamic range	0- ~400 pg/mL
Aβ42 Dynamic range	0- ~200 pg/mL
Aβ40 Dynamic range	0- ~600 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

***Plasma diluted 1:4 and CSF diluted 1:80. See Kit Instruction for details**

Endogenous Plasma Readings: Healthy donor EDTA plasma (n=10) samples were measured. Two Aβ42 samples read below the LOD and two Aβ40 samples read below the LLOQ. These samples were excluded from the mean concentration but were included in the median concentration. Bars depict median with interquartile range. Orange lines represent functional LLOQ.



Endogenous CSF Readings: CSF (n=10) samples were measured. Bars depict median with interquartile range. Orange lines represent functional LLOQ.



Target	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD
Tau	EDTA plasma	1.61	1.70	100%
	CSF	274	227	100%
Aβ42	EDTA plasma	3.95*	3.61	80%
	CSF	733	687	100%
Aβ40	EDTA plasma	72.0*	63.9	100%
	CSF	9845	10085	100%

*Values below LLOQ were not included in the mean

Precision: Measurements of 2 plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 3 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements).

Tau	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	2.00	3.4%	5.3%	1.3%
Control 2	88.5	3.6%	2.9%	3.2%
Panel 1	179	4.1%	2.9%	2.2%
Panel 2	5.82	3.5%	4.1%	2.2%

Aβ42	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	5.58	4.0%	2.6%	0.9%
Control 2	125	2.8%	2.3%	2.5%
Panel 1	8.49	4.0%	8.3%	4.6%
Panel 2	106	2.9%	4.0%	1.5%

Aβ40	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	17.6	3.3%	4.9%	4.7%
Control 2	246	2.2%	2.5%	2.6%
Panel 1	111	1.5%	4.4%	2.3%
Panel 2	74.0	1.3%	2.7%	1.2%

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

Tau	**Mean = 60%** Range: 37-81%
Aβ42	**Mean = 51%** Range: 38-60%
Aβ40	**Mean = 68%** Range: 57-79%

Dilution Linearity, Plasma: 2 spiked EDTA plasma samples were diluted 2x serially from 4x (MRD) to 32x with Sample Diluent.

Tau (32x)	**Mean = 140%** Range: 108-195%
Aβ42 (32x)	**Mean = 112%** Range: 107-118%
Aβ40 (32x)	**Mean = 104%** Range: 93-115%

Dilution Linearity, CSF: 1 spiked CSF sample was diluted 2X serially from 80x (MRD) to 640x with Sample Diluent.

Tau (640x)	**Mean = 103%** Range: 99-106%
Aβ42 (640x)	**Mean = 110%** Range: 104-113%
Aβ40 (640x)	**Mean = 78%** Range: 67-89%

The Simoa N3PA assay is formulated for use on either the SR-X or HD-1 platform. Data in this document was obtained from runs on the SR-X platform unless otherwise noted. Some differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two 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.

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