Quanterix™
The Science of Precision Health

Simoa® Neurology 3-Plex A Advantage Kit

HD-1/HD-X Data Sheet

Item 101995

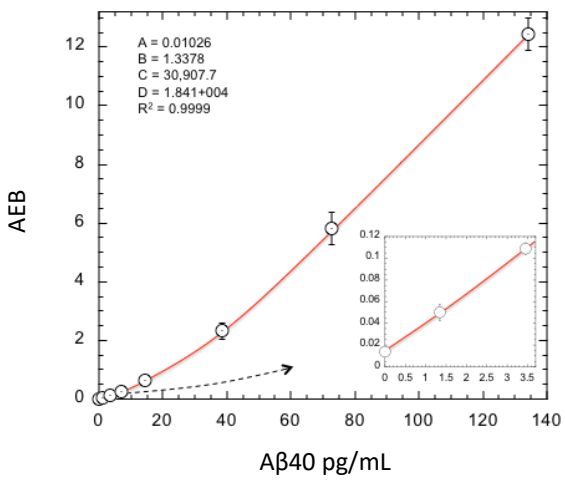
Neurology 3-Plex A (N3PA) Assay

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 are 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. Tau and amyloid β related pathologies have been tested and monitored as potential biomarkers for Alzheimer's disease, mild cognitive impairment, vascular dementia, and other neurodegenerative disorders.

Description – Aβ40

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.

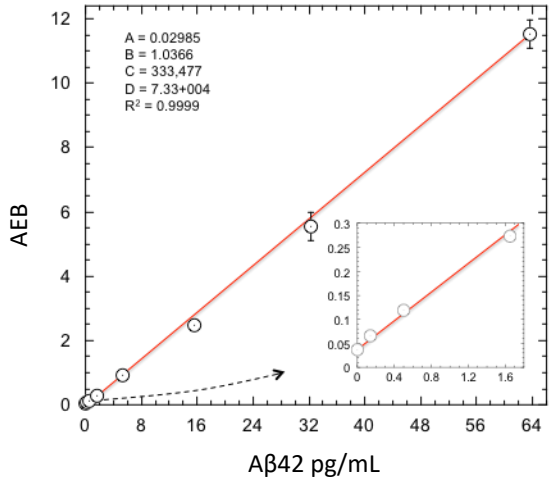
Calibration Curve: Four-parameter curve fit parameters are depicted.



Description – Aβ42

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, requiring high analytical sensitivity for its reliable measurement.

Calibration Curve: Four-parameter curve fit parameters are depicted.

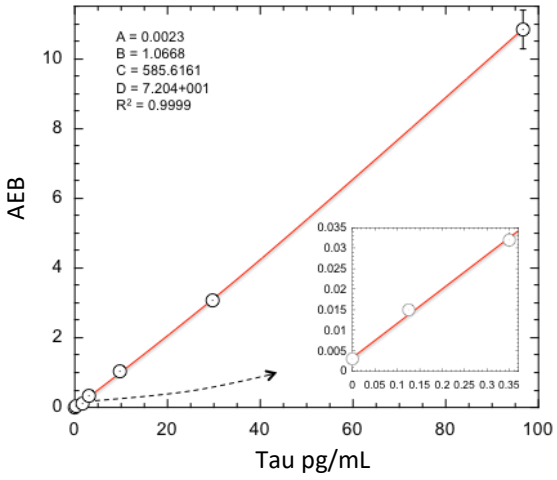


Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

Description – Tau

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 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.

Calibration Curve: Four-parameter curve fit parameters are depicted.



Minimum Required Dilution (MRD)

Diluted Sample Volume	152 µL per measurement
Plasma Dilution	1:4
CSF Dilution	1:80
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total). The Functional LLOQ for CSF is 80x Analytical LLOQ.

	Analytical LLOQ	Functional LLOQ (x MRD)
Aβ40	**0.675 pg/mL** pooled CV 12.5% mean recovery 107%	**2.70 pg/mL**
Aβ42	**0.142 pg/mL** pooled CV 15.6% mean recovery 109%	**0.568 pg/mL**
Tau	**0.063 pg/mL** pooled CV 9.1% mean recovery 107%	**0.252 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 2 reagent lots across 3 instruments (12 runs total).

	LOD
Aβ40	**0.196 pg/mL** range 0.044–0.372 pg/mL
Aβ42	**0.045 pg/mL** range 0.002–0.072 pg/mL
Tau	**0.019 pg/mL** range 0.004–0.044 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 plasma. The Upper Limit of Quantification (ULOQ) for CSF is 20x the ULOQ for plasma.

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

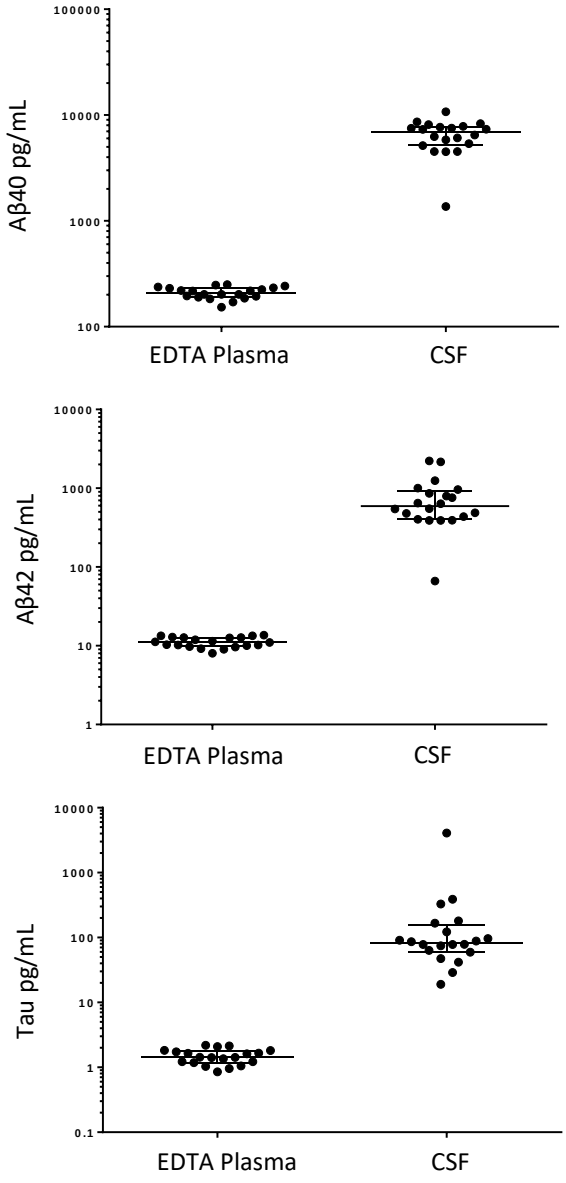
Simoa® Neurology 3-Plex A Advantage Kit

HD-1/HD-X Data Sheet

Item 101995

	Assay Range
Aβ40	0 – 560 pg/mL
Aβ42	0 – 240 pg/mL
Tau	0 – 400 pg/mL

Endogenous Sample Reading: Healthy donor EDTA plasma (n=20) and CSF (n=20) were measured. Error bars depict median with interquartile range.



	Sample Type	Median Conc pg/mL	% Above LOD
Aβ40	Plasma	209	100%
	CSF	6898	100%
Aβ42	Plasma	11.1	100%
	CSF	592	100%
Tau	Plasma	1.43	100%
	CSF	82.5	100%

Reproducibility Precision: Four samples consisting of two plasma panels and two calibrator-based controls were measured 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 tables below.

Aβ40	Mean (pg/mL)	Within run CV	Between run CV	Between lot CV	Between Inst CV
Control 1	22.4	3.4%	2.0%	0.0%	5.6%
Control 2	393	4.7%	0.0%	0.0%	2.0%
Panel 1	54.1	5.1%	0.0%	3.3%	0.0%
Panel 2	114	5.6%	1.7%	2.8%	0.0%

Aβ42	Mean (pg/mL)	Within run CV	Between run CV	Between lot CV	Between Inst CV
Control 1	3.20	5.5%	4.0%	0.0%	4.3%
Control 2	87.0	5.8%	1.9%	5.4%	0.0%
Panel 1	3.47	9.0%	0.0%	8.8%	0.0%
Panel 2	47.4	7.5%	0.0%	5.9%	0.0%

Tau	Mean (pg/mL)	Within run CV	Between run CV	Between lot CV	Between Inst CV
Control 1	2.24	5.8%	2.3%	5.8%	3.4%
Control 2	99.5	5.9%	3.8%	4.9%	0.0%
Panel 1	1.53	11.9%	0.0%	2.5%	8.9%
Panel 2	2.72	8.5%	4.5%	4.0%	2.5%

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

Repeatability Precision: Four samples consisting of two plasma panels and two calibrator-based controls were measured 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 tables below.

Aβ40	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	22.2	5.8%	1.8%	1.7%
Control 2	378	4.1%	4.9%	1.2%
Panel 1	52.9	3.5%	4.0%	1.7%
Panel 2	111	5.2%	3.3%	2.1%

Aβ42	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	3.12	8.1%	2.1%	0.0%
Control 2	84.4	5.5%	2.0%	2.0%
Panel 1	3.40	6.8%	0.0%	5.8%
Panel 2	45.3	6.7%	5.4%	2.3%

Tau	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	2.31	9.1%	0.0%	4.4%
Control 2	96.6	4.7%	2.2%	4.2%
Panel 1	1.51	7.8%	9.8%	0.0%
Panel 2	2.77	7.7%	4.9%	0.0%

Spike and Recovery: 4 CSF and 4 EDTA plasma samples were spiked with antigen at high and low concentrations within the range of the assay and measured.

	Plasma Recovery	CSF Recovery
Aβ40	**136%** range 59.8–331%	*
Aβ42	**63.1%** range 46.7–74.9%	**117%** range 84.6–142%
Tau	**61.3%** range 58.8–65.7%	**107%** range 96.3–123%

*High AB40 endogenous levels were observed in CSF samples; spiked AB40 samples exceeded dynamic range.

Dilution Linearity: 4 spiked EDTA plasma samples were diluted 2x serially from MRD (4x) to 16x with Sample Diluent and 1 spiked CSF sample was diluted 2x serially from MRD (80x) 10,240x with Sample Diluent.

	Plasma Linearity	CSF Linearity
Aβ40	**105%** range 91.1–116%	**83.0%** range 75.5–88.3%
Aβ42	**92.6%** range 70.8–110%	**98.3%** range 86.4–114%
Tau	**115%** range 101–138%	**101%** range 91.6–112%

The Simoa Neurology 3-Plex A 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.

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