Quanterix™
The Science of Precision Health

Simoa® Neurology 3-Plex A Kit (Tau, Aβ42, Aβ40)
HD-1/HD-X Data Sheet

Item 101995

Summary and Explanation of the Test

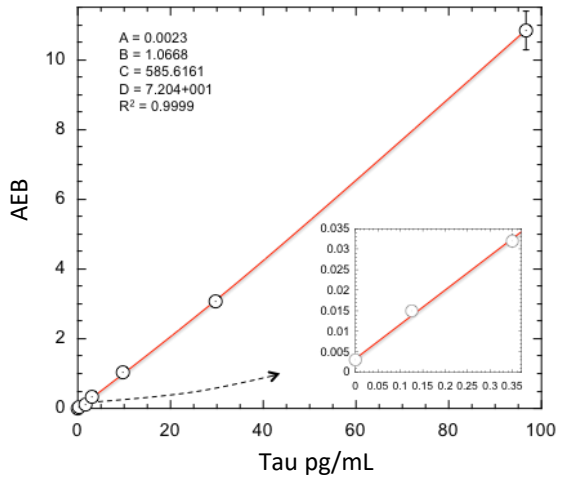
The Simoa Human N3PA assay is a digital immunoassay for the quantitative determination of total Tau, Aβ42, and Aβ40 in human plasma and CSF. Determination in serum samples are not reported due to high variability of Aβ40 and Aβ42 in some healthy donor sample sets. This assay is for research use only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

Tau 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. Aβ42 and Aβ40 are two proteolytic products from the amyloid precursor protein (APP). Beta-secretase cleavage of APP initially results in the production of an APP fragment that is further cleaved by gamma-secretase at residues 40 or 42 to generate two main forms of amyloid beta, Aβ42 and Aβ40. 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. Tau and amyloid β related pathologies have beenthe hallmark of Alzheimer's disease. CSF and blood tau and amyloid have been tested and monitored as potential biomarkers for Alzheimer's disease, mild cognitive impairment, vascular dementia, and other neurodegenerative disorders.

Description

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.

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



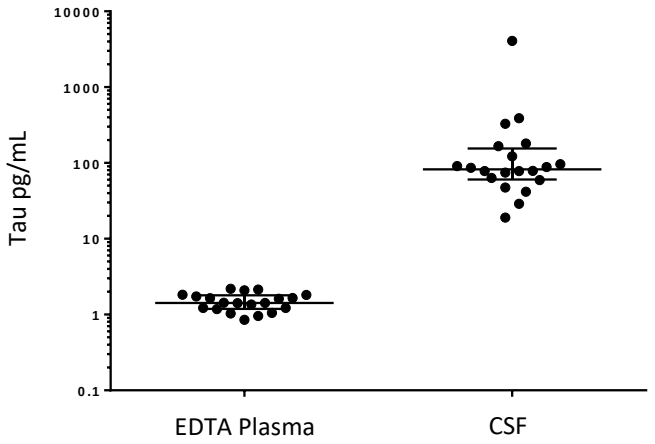
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total).

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).

LLOQ	**0.063 pg/mL** pooled CV 9.1% mean recovery 107%
LOD	**0.019 pg/mL** range 0.004–0.044 pg/mL
Dynamic range (serum and plasma)	0–400 pg/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Median Tau pg/mL	% Above LOD
CSF	82.5	100%
EDTA Plasma	1.43	100%

Reproducibility Precision: Four samples consisting of two plasma panels 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.

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

Repeatability Precision: Four samples consisting of two plasma panels 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.

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

Inter Lot CV: Pool of CVs from 6 samples (range: 2.3–19.6 pg/mL) tested with 2 reagent lots across 2 runs x 3 instruments.

Inter Instrument CV: Pool of CVs from 6 samples (range: range: 2.3–19.6 pg/mL) tested with 3 instruments across 2 runs x 2 reagent lots.

Inter Lot CV	**3.5%**
Inter Instrument CV	**4.1%**

Spike and Recovery (Plasma): Tau 441 spiked into 4 plasma samples at 20 and 200 pg/mL.

Spike and Recovery (CSF): Tau 411 spiked into 4 CSF samples at 400 and 4000 pg/mL.

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

Dilution Linearity (Plasma): Spiked plasma diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

Dilution Linearity (CSF): CSF diluted 2x serially from MRD (80x) to 10,240x with Sample Diluent.

Spike and Recovery (Plasma)	**Mean = 61.3%** Range: 58.8–65.7%
Spike and Recovery (CSF)	**Mean = 107 %** Range: 96.3–123%
Admixture Linearity	**Mean = 100%** Range: 88.1–108%
Dilution Linearity (Plasma - 64x)	**Mean = 115%** Range: 101–138%
Dilution Linearity (CSF - 10,240x)	**Mean = 101%** Range: 91.6–112%

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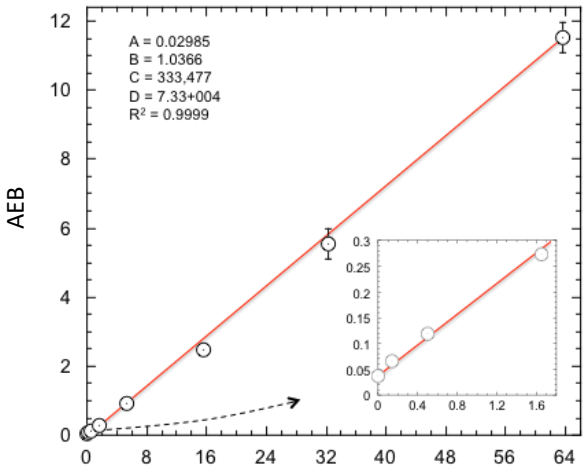
Description

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.



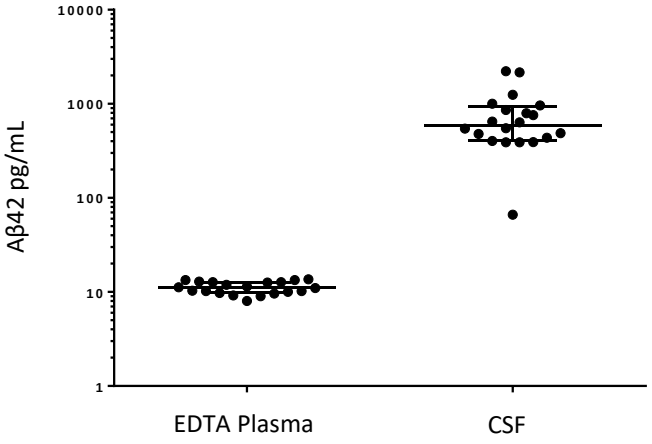
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total).

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).

LLOQ	**0.142 pg/mL** pooled CV 15.6% mean recovery 109%
LOD	**0.045 pg/mL** range 0.002–0.072 pg/mL
Dynamic range (serum and plasma)	0–240 pg/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Median Aβ42 pg/mL	% Above LOD
CSF	592	100%
EDTA Plasma	11.1	100%

Reproducibility Precision: Four samples consisting of two plasma panels and two Aβ42 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.

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

Repeatability Precision: Four samples consisting of two plasma panels and two Aβ42 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.

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

Inter Lot CV: Pool of CVs from 6 samples (range: 3.2–144 pg/mL) tested with 2 reagent lots across 2 runs x 3 instruments.

Inter Instrument CV: Pool of CVs from 6 samples (range: 3.2–144 pg/mL) tested with 3 instruments across 2 runs x 2 reagent lots.

Inter Lot CV	**3.5%**
Inter Instrument CV	**4.1%**

Spike and Recovery (Plasma): Aβ42 spiked into 4 plasma samples at 5 and 50 pg/mL.

Spike and Recovery (CSF): Aβ42 spiked into 4 CSF samples at 400 and 4000 pg/mL.

Admixture Linearity: High Aβ42 plasma sample fractionally admixed with low Aβ42 plasma sample, mean of 10 levels.

Dilution Linearity (Plasma): Spiked plasma diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

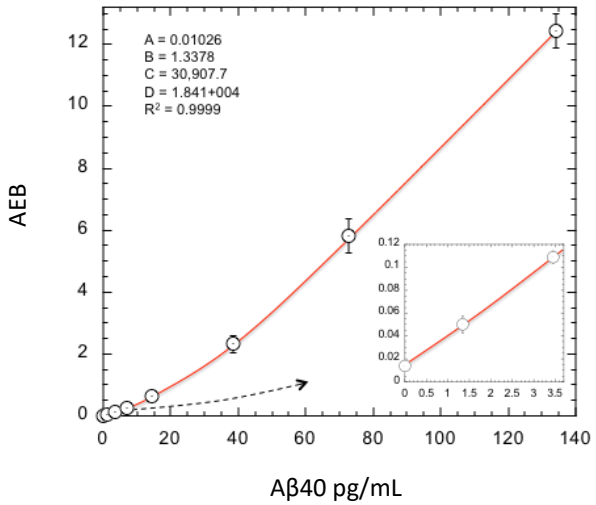
Dilution Linearity (CSF): CSF diluted 2x serially from MRD (80x) to 10,240x with Sample Diluent.

Spike and Recovery (Plasma)	**Mean = 63.1%** Range: 46.7–74.9%
Spike and Recovery (CSF)	**Mean = 117%** Range: 84.6–142%
Admixture Linearity	**Mean = 93.0%** Range: 72.2–104%
Dilution Linearity (Plasma - 64x)	**Mean = 92.6%** Range: 70.8–110%
Dilution Linearity (CSF – 10,240x)	**Mean = 98.3%** Range: 86.4–114%

Description

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.



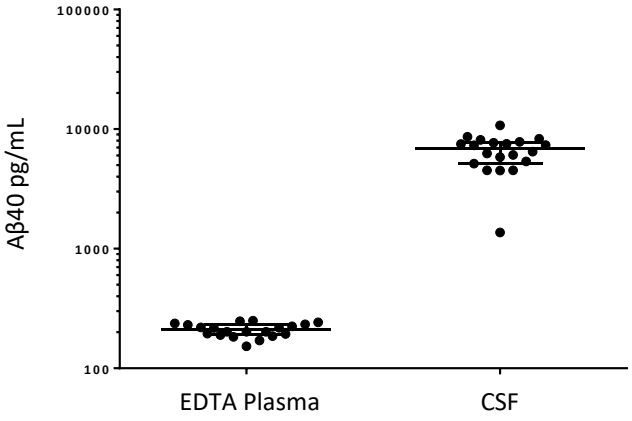
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total).

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).

LLOQ	**0.675 pg/mL** pooled CV 12.5% mean recovery 107%
LOD	**0.196 pg/mL** range 0.044–0.372 pg/mL
Dynamic range (serum and plasma)	0–560 pg/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Median Aβ40 pg/mL	% Above LOD
CSF	6898	100%
EDTA Plasma	209	100%

Reproducibility Precision: Four samples consisting of two plasma panels and two Aβ40 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.

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

Repeatability Precision: Four samples consisting of two plasma panels and two Aβ40 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.

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

Inter Lot CV: Pool of CVs from 6 samples (range: 22.6–389 pg/mL) tested with 2 reagent lots across 2 runs x 3 instruments.

Inter Instrument CV: Pool of CVs from 6 samples (range: 22.6–389 pg/mL) tested with 3 instruments across 2 runs x 2 reagent lots.

Inter Lot CV	**3.5%**
Inter Instrument CV	**4.1%**

Admixture Linearity: High Aβ40 serum sample fractionally admixed with low Aβ40 serum sample, mean of 10 levels.

Dilution Linearity (Plasma): Spiked plasma diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

Dilution Linearity (CSF): CSF diluted 2x serially from MRD (80x) to 10,240x with Sample Diluent.

Admixture Linearity	**Mean = 96.0%** Range: 89.2–112%
Dilution Linearity (Plasma - 64x)	**Mean = 105%** Range: 91.1–116%
Dilution Linearity (CSF – 10,240x)	**Mean = 83.0%** Range: 75.5–88.3%

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.