Simoa® Neurology 2-Plex A Advantage PLUS Kit

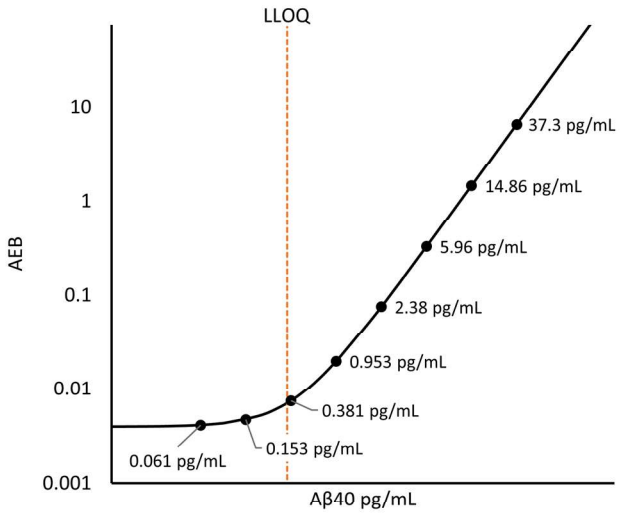
HD-X Data Sheet Item 104712

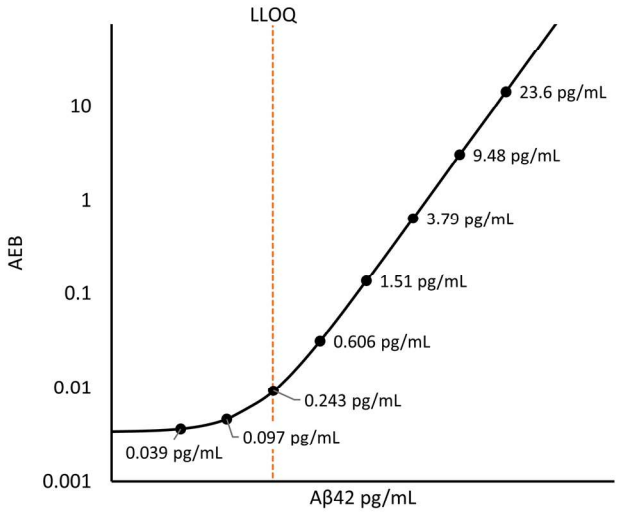
Description: This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the N2PA Advantage PLUS kit on the HD-X platform. Data provided includes Calibration Curves, Minimum Required Dilution (MRD), Limit of Detection (LOD), and Precision. As described in the Simoa NeuroPlex Advantage PLUS Assay Technical Note, additional relevant data provided is from the N4PE Advantage PLUS analytical validation.

A β 40: 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 EDTA 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.

A β 42: 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.

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





Minimum Required Dilution (MRD)

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

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): This data was obtained from the N4PE Advantage PLUS analytical validation. 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.

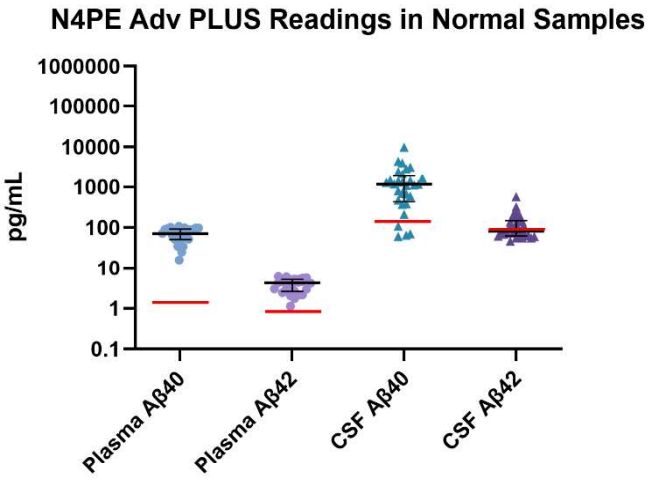
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 4 runs across 2 instruments (4 runs total).

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 EDTA plasma. The Upper Limit of Quantification (ULOQ) for CSF is 100x the ULOQ for EDTA plasma. Note that the top concentration will vary between kit lots, as calibrators are value assigned to maintain consistency of results across lots.

Aβ40	
Analytical LLOQ	**0.353 pg/mL** Pooled CV 9.4% Mean recovery 115%
Functional LLOQ	EDTA Plasma (4X): 1.41 pg/mL CSF (400X): 141 pg/mL
LOD	**0.262 pg/mL** Range:0.121–0.408 pg/mL
Dynamic Range	EDTA Plasma (4x): 0 – 180 pg/mL CSF (400x): 0 – 18 ng/mL

Aβ42	
Analytical LLOQ	**0.239 pg/mL** Pooled CV 15.1% Mean recovery 98.0%
Functional LLOQ	EDTA Plasma: 0.957 pg/mL CSF: 95.7 pg/mL
LOD	**0.111 pg/mL** Range:0.072–0.163 pg/mL
Dynamic Range	EDTA Plasma: 0 – 80 pg/mL CSF: 0 – 8 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.



Aβ40				
Sample Type	**Mean pg/mL**	**Median pg/mL**	**% Above LOD**	**% Above LLOQ**
EDTA Plasma	69.4	70.6	100	100
CSF	1838	1270	94.8	86.2

Aβ42				
Sample Type	**Mean pg/mL**	**Median pg/mL**	**% Above LOD**	**% Above LLOQ**
EDTA Plasma	3.80	4.16	100	100
CSF	197	158	86.2	46.6

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Precision: Measurements of 2 EDTA plasma-based panels, 2 CSF-based panels, and 2 calibrator-based controls. Triplicate measurements were made for 4 runs each for across 2 instruments (4 runs total, 12 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

Aβ40				
Sample	**Mean (pg/mL)**	**Within Run CV**	**Between Run CV**	**Between Instr CV**
Control 1	4.18	6.8%	7.3%	8.0%
Control 2	57.4	2.6%	23.3%	17.6%
Panel 1	2.50	8.7%	6.6%	6.0%
Panel 2	65.7	1.9%	8.0%	4.6%
Panel 3	5429	1.6%	3.4%	3.5%
Panel 4	9749	1.4%	3.9%	4.4%

Aβ42				
Sample	**Mean (pg/mL)**	**Within Run CV**	**Between Run CV**	**Between Instr CV**
Control 1	2.40	5.5%	10.5%	10.4%
Control 2	33.6	3.0%	20.8%	14.1%
Panel 1	1.49	7.6%	6.5%	7.8%
Panel 2	25.3	2.1%	8.6%	5.0%
Panel 3	404	4.7%	4.7%	4.6%
Panel 4	559	2.4%	7.5%	7.6%

Spike and Recovery: This data was obtained from the N4PE Advantage PLUS analytical validation. 2 EDTA plasma and 2 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 EDTA 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: This data was obtained from the N4PE Advantage PLUS analytical validation. 2 EDTA plasma and 2 CSF samples were serially diluted 2x with sample diluent and then tested at MRD. Total dilution of each sample ranged from 2x to 64x MRD and is reported as such. For valid comparison between

results, it is recommended to run all samples at a consistent dilution.

Aβ40	
Mean Spike and Recovery EDTA Plasma	**83.7%** Range: 83.5% – 84.0%
Mean Spike and Recovery CSF	**111%** Range: 107% - 114%
Mean Dilution Linearity Endogenous EDTA Plasma (2x - 32x MRD)	**92.7%** Range: 85.6% - 99.8%
Mean Dilution Linearity Endogenous CSF (2x - 64x MRD)	**87.7%** Range: 87.6%-87.9%

Aβ42	
Mean Spike and Recovery EDTA Plasma	**78.4%** Range: 77.7% - 79.2%
Mean Spike and Recovery CSF	**106%** Range: 104% - 109%
Mean Dilution Linearity Spiked EDTA Plasma (2x - 32x MRD)	**103%** Range: 101%-105%
Mean Dilution Linearity Spiked CSF (2x - 64x MRD)	**103%** Range: 102%-104%

The Simoa N2PA 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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