Quanterix
Discovery Fueled by Ultra-Sensitivity

Simoa® GFAP Advantage PLUS Kit

HD-X Data Sheet Item 104691

Description: This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the GFAP 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 here is from the N4PE Advantage PLUS analytical validation.

GFAP: 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.

Calibration Curve: Reconstitution volume of the calibrator concentrate is value assigned on a kit lot-specific basis. An example of calibrator concentrations and Lower Limit of Quantification (LLOQ) are depicted in Figure 1.

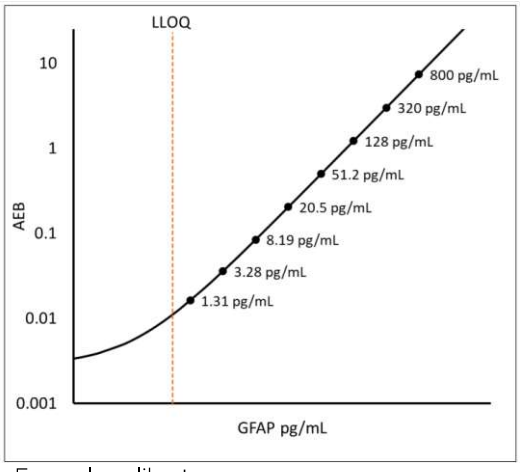

Figure 1. Example calibrator curve.

Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
EDTA Plasma/Serum 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 and serum. The Upper Limit of Quantification (ULOQ) for CSF is 100x the ULOQ for EDTA plasma/Serum. Note that the top concentration will vary between kit lots, as Calibrators are value assigned to maintain consistency of results across lots.

Analytical LLOQ	**0.635 pg/mL** Pooled CV 15.5% Mean recovery 97.6%
Functional LLOQ	EDTA Plasma/Serum (4X): 2.54 pg/mL CSF (400X): 254 pg/mL
LOD	**0.138 pg/mL** Range: 0.017 – 0.429 pg/mL
Dynamic Range	EDTA Plasma/Serum (4x): 0 – 3200 pg/mL CSF (400x): 0 – 320 ng/mL

Endogenous Sample Reading: Concentrations (pg/mL) were determined for EDTA plasma (n=26), serum (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.

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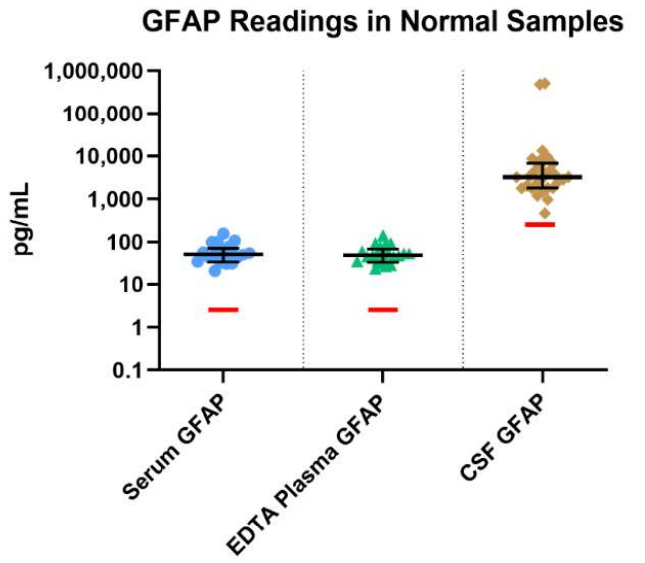

Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
EDTA Plasma	80.4	68.5	100%	100%
Serum	59.2	51.3	100%	100%
CSF	37858	3239	100%	100%

Precision: Measurements of 2 EDTA plasma-based panels, 1 serum-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.

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV
Control 1	90.3	5.8%	7.2%	6.5%
Control 2	1101	5.9%	6.0%	2.7%
Panel 1	22.9	5.7%	7.2%	4.8%
Panel 2	856	3.3%	12.6%	7.6%
Panel 3	7299	5.7%	11.2%	7.4%
Panel 4	4715	5.7%	12.4%	13.4%
Panel 5	98.8	3.7%	16.8%	0.2%
Panel 6	490	6.4%	10.8%	4.6%

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, 2 serum and 2 CSF samples were serially diluted 2x with sample diluent and then tested at MRD. Total dilution of each EDTA plasma/serum sample ranged from4x (MRD) to 64x. Total dilution of each CSF sample ranged from 400x (MRD) to 51200x. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

Mean Spike and Recovery Plasma	**71.7%** Range: 71.6% - 71.7%
Mean Spike and Recovery Serum	**89.6%** Range: 80.1 - 106.8%
Mean Spike and Recovery CSF	**106%** Range: 105% - 108%
Mean Dilution Linearity Endogenous EDTA Plasma	**106%** Range: 105% - 107%
Mean Dilution Linearity Endogenous Serum	**122.4%** Range: 112.1 - 136.7%
Dilution Linearity Endogenous CSF	**94.8%** Range: 94.1% - 95.5%

The Simoa GFAP 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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