

This study report demonstrates the Simoa N2PB Advantage PLUS assay properties on Simoa HD-X for the generation of claims data. As discussed on the Simoa NeuroPlex Advantage PLUS Assay Technical Note, Products Data provided in Sections I – III includes Limit of Detection (LOD) and Precision. Performance comparison between Simoa N2PB Advantage PLUS and N4PE Advantage PLUS assay is provided in Sections IV and V including the comparison on LOD, LLOQ, Precision and Sample Measurement Bias.

I. N2PB Advantage PLUS Kit Summary

NF-L

Test	Claims Yield from N2PB Advantage PLUS Validation
LOD	0.080 ± 0.044 pg/mL Range: 0.033 – 0.119 pg/mL
Calibrator B/A	3.57 ± 0.450 Range: 3.08 – 4.02
Calibrator A	0.009 ± 0.0006 Range: 0.009 – 0.010
Calibrator I	12.7 ± 0.431 Range: 12.3 – 13.3
Precision	Mean Total CV: 5.3% Within Run CV: 4.7% Between Run CV: 5.2% Between Instrument CV: 6.1%

Test	Claims Reference from N4PE Advantage PLUS Validation Report
Analytical LLOQ	0.402 pg/mL
Functional LLOQ	EDTA Plasma: (4x): 1.61 pg/mL CSF: (400x): 161 pg/mL
Spike and Recovery	EDTA Plasma: 96.0%, Range: 93.2% - 98.8% CSF: 105%, Range: 103% - 107%
Dilution Linearity	EDTA Plasma (spiked; 4x (MRD) - 512x): 92.8%, Range: 91.1% - 94.6% CSF (spiked; 400x (MRD) - 51200x): 93.7%, Range: 93.0% - 94.3%
Curve Storage	Bias: 4.4 %, Range: 3.3% - 6.4%
Drift	Three-plate Variance Plate 1: 5.7%; Plate 1+2: 5.2%; Plate 1+2+3: 5.8% Three-plate Precision: 4.5%, Range: 3.6% - 5.3%
Normal Samples *	EDTA Plasma Mean: 9.40 pg/mL, Range: 2.75 – 27.0 pg/mL Detectability: 100%, Quantifiability: 100% CSF Mean: 3051pg/mL, Range: 182 - 20211 pg/mL Detectability: 100%, Quantifiability: 100%

* Values below LLOQ are not included in the mean.

GFAP

Test	Claims Yield from N2PB Advantage PLUS Validation
LOD	0.111 ± 0.049 pg/mL Range: 0.058 – 0.172 pg/mL
Calibrator B/A	6.15 ± 0.686 Range: 5.66 – 7.15
Calibrator A	0.003 ± 0.0003 Range: 0.003 – 0.004
Calibrator I	9.51 ± 0.606 Range: 8.96 – 10.3
Precision	Mean Total CV: 7.4% Within Run CV: 4.2% Between Run CV: 8.1% Between Instrument CV: 9.8%

Test	Claims Reference from N4PE Advantage PLUS Validation
Analytical LLOQ	0.635 pg/mL
Functional LLOQ	EDTA Plasma: (4x): 2.54 pg/mL CSF: (400x): 254 pg/mL
Spike and Recovery	EDTA Plasma: 71.7%, Range: 71.6% - 71.7% CSF: 106%, Range: 105% - 108%
Dilution Linearity	EDTA Plasma (endogenous; 4x (MRD) - 64x): 106%, Range: 105% - 107% CSF (endogenous; 400x (MRD) - 51200x): 94.8%, Range: 94.1% - 95.5%
Curve Storage	Bias: 7.3%, Range: 5.2% - 12.0%
Drift	Three-plate Variance Plate 1: 9.5%; Plate 1+2: 13.4%; Plate 1+2+3: 14.8% Three-plate Precision: 6.2%, Range: 4.8% - 8.4%
Normal Samples *	EDTA Plasma Mean: 80.4 pg/mL, Range: 31.0 – 225 pg/mL, Detectability: 100%, Quantifiability: 100% CSF Mean: 37858 pg/mL, Range: 461 – 506642 pg/mL, Detectability: 100%, Quantifiability: 100%

* Values below LLOQ are not included in the mean.

II. NFL Individual Claim Summaries

Limit of Detection:

The table below shows the calculated LOD (Lower Limit of Detection). LOD is calculated as 2.5 Standard Deviations above the background (mean of calibrator blanks).

Test Conditions		Claim
Replicates/Run (each test sample)	3	Grand Mean LOD: 0.080 pg/mL Range: 0.033 – 0.119 pg/mL
# Instruments	2	
Runs/Instrument	2	
# Lots Tested	1	
Total Runs	4	

Precision Profile:

2 Plasma Panels and 2 controls were diluted 1:4 in plasma sample diluent. 2 CSF Panels were diluted 1:400 in CSF sample diluent. 4 runs were run on 2 instruments. The %CVs based on concentration are reported in the table below.

Test Conditions		Claim
Test Samples	Control 1: Low Concentration Calibrator spiked into calibrator diluent Control 2: High Concentration Calibrator spiked into calibrator diluent Panel 1: Normal Human EDTA Plasma Sample spiked with antigen Panel 2: Normal Human EDTA Plasma Sample spiked with antigen Panel 3: Normal Human CSF Sample Panel 4: Normal Human CSF Sample	Mean Total CV: 5.3%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 2 Lots Tested: 1 Total Runs: 4; Total Measurements/Sample: 12	
Within Run CV		4.7%
Between Run CV		5.2%
Between Instrument CV		6.1%

III. GFAP Individual Claim Summaries

Limit of Detection:

The table below shows the calculated LOD (Lower Limit of Detection). LOD is calculated as 2.5 Standard Deviations above the background (mean of calibrator blanks).

Test Conditions		Claim
Replicates/Run (each test sample)	3	Grand Mean LOD: 0.111 pg/mL Range: 0.058 – 0.172 pg/mL
# Instruments	2	
Runs/Instrument	2	
# Lots Tested	1	
Total Runs	4	

Precision Profile:

2 EDTA Plasma Panels and 2 controls were diluted 1:4 in plasma sample diluent. 2 CSF Panels were diluted 1:400 in CSF sample diluent. 4 runs were run on 2 instruments. The %CVs based on concentration are reported in the table below.

Test Conditions		Claim
Test Samples	Control 1: Low Concentration Calibrator spiked into calibrator diluent Control 2: High Concentration Calibrator spiked into calibrator diluent Panel 1: Normal Human EDTA Plasma Sample spiked with antigen Panel 2: Normal Human EDTA Plasma Sample spiked with antigen Panel 3: Normal Human CSF Sample Panel 4: Normal Human CSF Sample	Mean Total CV: 7.4%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 2 Lots Tested: 1 Total Runs: 4; Total Measurements/Sample: 12	
Within Run CV		4.2%
Between Run CV		8.1%
Between Instrument CV		9.8%

IV. NF-L Claims Detail

LOD Summary

N2PB Advantage PLUS Kit Assay Characterization		
LOD	0.080 ± 0.044 (0.033 – 0.119)	n=4, Mean of 2.5 SD LOD calculated from calibration curves, 1 lot tested across 2 instrument, 4 runs total
Cal B/Cal A	3.57 ± 0.450 (3.08 – 4.02)	n=4, Mean of Cal B/Cal A calculated from calibration curves, 1 lot tested across 2 instruments, 4 runs total
Cal A	0.009 ± 0.0009 (0.009 – 0.010)	n=4, Mean of Cal A from calibration curves, 1 lot tested across 2 instruments, 4 runs total
Cal I	12.7 ± 0.431 (12.3 – 13.3)	n=4, Mean of Cal I from calibration curves, 1 lot tested across 2 instruments, 4 runs total

Comparing NF-L in N2PB Advantage PLUS LOD with NF-L in N4PE Advantage PLUS LOD

NF-L in N2PB Advantage PLUS							NF-L in N4PE Advantage PLUS	
Run	1	2	3	4	AVE	SD	AVE	SD
LOD pg/mL	0.033	0.119	0.116	0.052	0.080	0.044	0.072	0.054
Cal B/A	4.02	3.08	3.89	3.31	3.57	0.450	4.44	0.471
Cal A	0.009	0.010	0.009	0.009	0.009	0.0006	0.010	0.0009
Cal I	13.3	12.3	12.6	12.7	12.7	0.431	12.9	0.570

Analytical LLOQ

Recovery %				
Run	1	2	3	4
2.10 pg/mL	104%	98.0%	104%	103%
1.52 pg/mL	100%	101%	108%	115%
0.839 pg/mL	101%	99.2%	103%	93.7%
0.434 pg/mL	58.8%	67.6%	123%	137%
0.216 pg/mL	125%	150%	166%	142%
Pooled CV				
Run	1	2	3	4
2.10 pg/mL	4.7%	8.5%	8.1%	4.1%
1.52 pg/mL	4.4%	17.3%	5.4%	2.9%
0.839 pg/mL	13.1%	8.5%	3.6%	2.3%
0.434 pg/mL	13.0%	26.9%	25.7%	0.5%
0.216 pg/mL	9.6%	4.6%	8.1%	11.2%

Comparing NF-L in N2PB Advantage PLUS LLOQ with NF-L in N4PE Advantage PLUS LLOQ

NF-L in N2PB Advantage PLUS			NF-L in N4PE Advantage PLUS		
LLOQ Target (pg/ml)	Pooled CV	Mean Recovery	LLOQ Target (pg/ml)	Pooled CV	Mean Recovery
2.10	7.7%	102%	2.01	5.0%	99.2%
1.52	10.9%	108%	1.41	9.5%	91.3%
0.839	9.3%	99.4%	0.804	8.6%	101%
0.434*	22.8%	96.5%	0.402	15.3%	97.7%
0.216	10.1%	146%	0.201	22.4%	112%

* Data derived from limited runs (n=4) as compared to the parent study for N4PE (n=12). Observed difference of LLoQ for derivative assays is due to a combination of study design, lower *n* and Bessel's correction used for the calculation of SDs.

Comparing NF-L in N2PB Advantage PLUS Precision with NF-L in N4PE Advantage PLUS Precision

NF-L in N2PB Advantage PLUS					NF-L in N4PE Advantage PLUS				
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV	Between lot CV
Control 1	89.0	3.6%	2.4%	2.4%	81.8	2.6%	9.8%	1.9%	1.0%
Control 2	1006	2.1%	4.1%	5.0%	1079	3.7%	7.2%	0.0%	3.4%
Panel 1**	5.23	7.1%	11.2%	13.2%	51.3	2.7%	7.0%	3.0%	0.0%
Panel 2**	453	3.6%	5.8%	7.0%	641	2.9%	8.1%	1.6%	3.5%
Panel 3**	25473	5.5%	3.9%	4.2%	19964	3.9%	8.8%	2.2%	1.2%
Panel 4**	1838	6.0%	3.7%	4.5%	1441	6.3%	10.3%	3.9%	1.1%

** Panels used for N2PB Advantage PLUS Validation are not identical to panels used for N4PE Advantage PLUS Validation

Comparing NF-L in N2PB Advantage PLUS Sample Measurement with NF-L in N4PE Advantage PLUS

Spiked EDTA Plasma Sample	NF-L in N2PB Advantage PLUS 4 Run Mean (pg/mL)	NF-L in N4PE Advantage PLUS 4 Run Mean (pg/mL)	Bias
1	7.20	7.02	2.6%
2	2.08	2.18	-4.6%
3	5.23	5.18	1.0%
4	3.75	3.49	7.2%
5	5.93	5.77	2.8%
6	3.32	3.30	0.6%
7	4.49	4.57	-1.7%
8	6.20	6.01	3.0%
9	50.1	51.4	-2.6%
10	53.6	57.3	-6.4%
11	453	463	-2.0%
12	427	439	-2.7%
13	2.42	2.40	0.7%
14	6.28	6.70	-6.3%
15	3.78	4.06	-7.1%
16	4.72	4.77	-1.1%
17	4.28	4.17	2.6%
18	12.7	12.6	0.6%
19	3.72	4.09	-9.1%
20	5.21	5.19	0.3%
21	57.7	59.6	-3.2%
22	50.9	50.3	1.1%
23	448	443	1.1%
24	470	454	3.4%
Mean Bias		-0.8%	

V. GFAP Claims Detail

LOD Summary

GFAP Advantage PLUS Kit Assay Characterization		
LOD	0.111 ± 0.049 (0.058 – 0.172)	n=4, Mean of 2.5 SD LOD calculated from calibration curves, 1 lot tested across 2 instruments per lot, 12 runs total
Cal B/Cal A	6.15 ± 0.686 (5.66 – 7.15)	n=4, Mean of Cal B/Cal A calculated from calibration curves, 1 lot tested across 2 instruments, 4 runs total
Cal A	0.003 ± 0.0003 (0.003 – 0.004)	n=4, Mean of Cal A from calibration curves, 1 lot tested across 2 instruments, 4 runs total
Cal I	12.7 ± 0.431 (12.3 – 13.3)	n=4, Mean of Cal I from calibration curves, 1 lot tested across 2 instruments per lot, 12 runs total

Comparing GFAP in N2PB Advantage PLUS LOD with GFAP in N4PE Advantage PLUS LOD

GFAP in N2PB Advantage PLUS							GFAP in N4PE Advantage PLUS	
Run	1	2	3	4	AVE	SD	AVE	SD
LOD pg/mL	0.058	0.089	0.172	0.123	0.1111	0.049	0.139	0.080
Cal B/A	5.66	5.74	6.04	7.15	6.15	0.686	7.28	0.706
Cal A	0.004	0.003	0.003	0.003	0.003	0.0003	0.004	0.0003
Cal I	10.3	9.14	8.96	9.62	9.51	0.606	11.1	0.670

Analytical LLOQ

Recovery %				
Run	1	2	3	4
2.36 pg/mL	104%	99%	108%	100%
2.24 pg/mL	109%	118%	107%	111%
0.944 pg/mL	98.3%	101%	99.3%	103%
0.642 pg/mL	61.0%	59.0%	122%	109%
0.321 pg/mL	125%	155%	156%	127%
Pooled CV				
Run	1	2	3	4
2.36 pg/mL	3.7%	4.9%	3.1%	1.9%
2.24 pg/mL	5.2%	5.7%	3.8%	5.3%
0.944 pg/mL	4.5%	8.1%	6.8%	2.2%
0.642 pg/mL	2.3%	22.6%	5.6%	8.3%
0.321 pg/mL	31.2%	6.5%	14.2%	9.5%

Comparing GFAP in N2PB Advantage PLUS LLOQ with GFAP in N4PE Advantage PLUS LLOQ

GFAP in N2PB Advantage PLUS			GFAP in N4PE Advantage PLUS		
LLOQ Target (pg/ml)	Pooled CV	Mean Recovery	LLOQ Target (pg/ml)	Pooled CV	Mean Recovery
2.36	4.1%	103%	3.18	7.1%	100%
2.24	5.8%	112%	2.22	7.5%	109%
0.944	6.8%	100%	1.27	9.8%	103%
0.642*	14.3%	87.6%	0.635	15.5%	97.6%
0.321	20.9%	141%	0.318	24.1%	113%

* Data derived from limited runs (n=4) as compared to the parent study for N4PE (n=12). Observed difference of LLoQ for derivative assays is due to a combination of study design, lower *n* and Bessel's correction used for the calculation of SDs.

Comparing GFAP in N2PB Advantage PLUS Precision with GFAP in N4PE Advantage PLUS Precision

GFAP in N2PB Advantage PLUS					GFAP in N4PE Advantage PLUS				
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV	Between lot CV
Control 1	89.6	4.2%	5.2%	6.2%	119	3.2%	10.5%	1.4%	0.3%
Control 2	1176	2.5%	5.2%	6.1%	1531	2.8%	6.3%	1.5%	3.0%
Panel 1**	22.6	6.7%	7.7%	9.4%	53.8	3.8%	9.5%	0.6%	2.7%
Panel 2**	857	4.3%	11.0%	13.3%	1184	3.3%	8.9%	2.1%	3.8%
Panel 3**	7291	4.4%	4.4%	5.3%	7044	2.7%	12.7%	2.1%	4.7%
Panel 4**	4435	3.3%	15.4%	18.7%	4053	3.1%	14.6%	2.2%	0.5%

** Panels used for N2PB Advantage PLUS Validation are not identical to panels used for N4PE Advantage PLUS Validation

Comparing GFAP in N2PB Advantage PLUS Sample Measurement with GFAP inN4PE Advantage PLUS

Spiked EDTA Plasma Sample	GFAP in N2PB Advantage PLUS 4 Run Mean (pg/mL)	GFAP inN4PE Advantage PLUS 4 Run Mean (pg/mL)	Bias
1	5.61	5.72	-1.8%
2	18.4	18.7	-1.5%
3	22.6	22.6	0.0%
4	25.4	25.3	0.4%
5	27.7	27.6	0.2%
6	29.1	28.8	0.8%
7	29.4	29.3	0.2%
8	30.2	28.2	7.1%
9	44.0	44.4	-1.0%
10	43.7	44.3	-1.4%
11	857	858	0.0%
12	997	1007	-1.0%
13	33.1	32.6	1.3%
14	32.9	34.9	-5.7%
15	33.3	33.7	-1.2%
16	33.7	34.6	-2.6%
17	33.7	33.5	0.5%
18	35.6	35.4	0.6%
19	35.8	35.9	-0.2%
20	26.3	26.6	-1.1%
21	57.1	57.1	-0.1%
22	86.2	84.2	2.3%
23	817	770	6.1%
24	742	729	1.8%
Mean Bias			0.2%