

This study report demonstrates the Simoa Neurology 4-Plex E (N4PE) Advantage assay properties on the Simoa HD-X for the generation of claims data. The N4PE assay measures Abeta 40 (Aβ40), Abeta 42 (Aβ42), Glial Fibrillary Acidic Protein (GFAP), and Neurofilament light (NF-L) in human EDTA plasma and CSF. Data provided here includes Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), spike and recovery, dilution linearity, curve storage, drift, precision, and normal sample range.

## I. Neurology 4-Plex E Advantage Kit Summary

### Aβ40

Test	Claim
LOD	0.384 ± 0.114 pg/mL (0.189 – 0.531 pg/mL)
Analytical LLOQ (Fit for Purpose)	1.02 pg/mL
Functional LLOQ	EDTA Plasma (4x): 4.08 pg/mL CSF (400x): 408 pg/mL
Calibrator B/A	4.14 ± 0.772 Range: 3.18-5.54
Calibrator A	0.004 ± 0.001 Range: 0.003-0.005
Calibrator H	11.4 ± 0.860 Range: 10.5-13.0
Precision	Within Run CV: 2.4% Between Run CV: 3.1% Between Instrument CV: 0.55% Between Lot CV: 1.7%
Spike and Recovery	EDTA Plasma: 67.8%, Range: 61.8-72.9% CSF: 94.9%, Range: 86.7-106%
Dilution Linearity	Endogenous EDTA Plasma (8x): 112%, Range: 103-125% Spiked EDTA Plasma (32x): 101%, Range: 89.3-115% Endogenous CSF (800x): 92%, Range: 87.9-97.2% Spiked CSF (12800x): 88%, Range: 78.8-101%
Curve Storage	Bias: 1.22%, Range: 0.96-1.5%
Drift	Three-plate Variance: Plate 1: 7.5%, Plate 1+2: 5.0%, Plate 1+2+3: 4.9% Three-plate Precision: 3.5%, Range: 2.6–4.5%
Normal Samples	EDTA Plasma: 90.9 pg/mL, Range: 69.1-112 pg/mL, Detectability: 100%, Quantifiability: 100% CSF: 4862 pg/mL, Range: 79.7 -13899 pg/mL, Detectability: 96%, Quantifiability: 96%
Kit Stability	6 months

**Aβ42**

Test	Claim
LOD	0.136 ± 0.054 (0.0601 - 0.204 pg/mL)
Analytical LLOQ (Fit for Purpose)	0.378 pg/mL
Functional LLOQ	EDTA Plasma (4x): 1.51 pg/mL CSF (400x): 151 pg/mL
Calibrator B/A	3.25 ± 0.490 Range: 2.60-4.07
Calibrator A	0.004 ± 0.001 Range: 0.003-0.004
Calibrator H	13.0 ± 0.824 Range: 11.9-14.5
Precision	Within Run CV: 3.4% Between Run CV: 5.5% Between Instrument CV: 1.0% Between Lot CV: 2.5%
Spike and Recovery	EDTA Plasma: 71.7%, Range: 65.4 – 75.7% CSF: 90.8%, Range: 85.4 – 95.0%
Dilution Linearity	Endogenous EDTA Plasma (8x): 93%, Range: 85.6-100% Spiked EDTA Plasma (32x): 101%, Range: 90.1-123% Endogenous CSF (800x): 99%, Range: 96.1-101% Spiked CSF (12800x): 107%, Range: 89.7-158%
Curve Storage	Bias: 0.91%, Range: 0.63 – 1.3%
Drift	Three-plate Variance: Plate 1: 8.7%, Plate 1+2: 4.6%, Plate 1+2+3: 5.8% Three-plate Precision: 3.8%, Range: 2.4-4.8%
Normal Samples	EDTA Plasma: 5.87 pg/mL, Range: 4.49-7.85 pg/mL, Detectability: 100%, Quantifiability: 100% CSF: 321 pg/mL, Range: 79.6-1025 pg/mL, Detectability: 100%, Quantifiability: 80%
Kit Stability	6 months

**GFAP**

Test	Claim
LOD	0.441 ± 0.179 (0.240 – 0.757 pg/mL)
Analytical LLOQ (Fit for Purpose)	2.89 pg/mL
Functional LLOQ	EDTA Plasma (4x): 11.6 pg/mL CSF (400x): 1156 pg/mL
Calibrator B/A	7.66 ± 0.687 Range: 6.43-9.13
Calibrator A	0.003 ± 0.0003 Range: 0.003-0.004
Calibrator H	14.2 ± 1.205 Range: 12.5-16.3
Precision	Within Run CV: 5.1% Between Run CV: 8.6% Between Instrument CV: 2.2% Between Lot CV: 4.3%
Spike and Recovery	EDTA Plasma: 58.1%, Range: 49.5 – 64.8% CSF: 91.4%, Range: 84.4 – 96.7%
Dilution Linearity	Endogenous EDTA Plasma (8x): 117%, Range: 108-129% Spiked EDTA Plasma (32x): 118%, Range: 105-140% Endogenous CSF (800x): 102%, Range: 99.9-104% Spiked CSF (12800x): 93%, Range: 76.9-102%
Curve Storage	Bias: 6.38%, Range: 5.31 - 8.1%
Drift	Three-plate Variance: Plate 1: 12.4%, Plate 1+2: 9.6%, Plate 1+2+3: 5.9% Three-plate Precision: 7.89%, Range: 4.2 - 8.7%
Normal Samples	EDTA Plasma: 58.8 pg/mL, Range: 19.9-181 pg/mL, Detectability: 100%, Quantifiability: 100% CSF: 17426 pg/mL, Range: 478-94189 pg/mL, Detectability: 100 %, Quantifiability: 92%
Kit Stability	6 months

**NF-L**

Test	Claim
LOD	0.09 ± 0.050 (0.016 – 0.152 pg/mL)
Analytical LLOQ (Fit for Purpose)	0.400 pg/mL
Functional LLOQ	EDTA Plasma (4x): 1.6 pg/mL CSF (400x): 160 pg/mL
Calibrator B/A	2.48 ± 0.278 Range: 2.25-3.25
Calibrator A	0.011 ± 0.002 Range: 0.007-0.013
Calibrator H	11.6 ± 0.884 Range: 10.5-12.8
Precision	Within Run CV: 6.0% Between Run CV: 7.3% Between Instrument CV: 2.5% Between Lot CV: 4.4%
Spike and Recovery	EDTA Plasma: 111%, Range: 94.9-120% CSF: 88.7%, Range: 80.6-93.4%
Dilution Linearity	Endogenous EDTA Plasma (8x): 111%, Range: 91.2-118% Spiked EDTA Plasma (32x): 97%, Range: 86-104% Endogenous CSF (800x): 91%, Range: 86.6-96% Spiked CSF (12800x): 92%, Range: 83.3-100%
Curve Storage	Bias: 2.36%, Range: 1.6 - 3.3%
Drift	Three-plate Variance: Plate 1: 13.6%, Plate 1+2: 11.1%, Plate 1+2+3: 11.2% Three-plate Precision: 8.58%, Range: 4.5 – 9.1%
Normal Samples	EDTA Plasma: 13.6 pg/mL, Range: 2.80-49.0 pg/mL, Detectability: 100%, Quantifiability: 100%, CSF: 2743 pg/mL, Range: 249-25722 pg/mL, Detectability: 100%, Quantifiability: 100%
Kit Stability	6 months

## II. Aβ40 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: 0.384 pg/mL  Range: 0.189 - 0.531 pg/mL
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

### Limit of Quantification:

The table below shows the calculated LLOQ (Lower Limit of Quantification). Analytical LLOQ is the lowest concentration of calibrator with ≤ 20% pooled CV and 80 – 120% mean recovery over all runs. Functional LLOQ is the Analytical LLOQ multiplied by the Minimum Required Dilution.

Test Conditions		Claim
Samples	Calibrator B at 1.130 pg/mL and diluted to 0.563 pg/mL Calibrator C at 2.03 pg/mL and diluted to 1.02 pg/mL and 0.508 pg/mL	Analytical LLOQ: 1.02 pg/mL  Functional LLOQ (EDTA Plasma) (4x) 4.08 pg/mL  Functional LLOQ (CSF) (400x) 408 pg/mL
Replicates/Run (each test sample)	3	
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Precision Profile:**

3 Plasma Panels and 2 controls were diluted 1:4 in Plasma Sample Diluent and 1 CSF Panel was diluted 1:400 in CSF Sample Diluent. 12 runs were run on 2 instruments comparing 2 reagent lots. The mean concentration corrected for MRD (pg/mL) and % CV 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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator Panel 4: Normal Human CSF Sample	Mean Total CV: 1.9%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 3 Lots Tested: 2 Total Runs: 12; Total Measurements/Sample: 36	
Within Run CV		2.4%
Between Run CV		3.1%
Between Instrument CV		0.55%
Between Lot CV		1.7%

**Spike Recovery (Plasma):**

2 normal plasma samples and a Diluent Control were spiked at two concentrations (high and low) with Aβ40 antigen and then diluted 1:4 with Sample Diluent. Percent recovery is defined as the measured concentration of Aβ40 in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of Aβ40 in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples– 2 EDTA plasma	Buffer Corrected Grand mean: 67.8% Range: 61.8% - 72.9%
Spike Concentrations	Control: Calibrator Diluent Low: 50 pg/mL High: 175 pg/mL	
Replicates/Run (each test sample)	3	

**Spike Recovery (CSF):**

2 normal CSF samples and a Diluent Control were diluted 1:400 in Sample diluent and then spiked at two concentrations (high and low) with Aβ40 antigen. Percent recovery is defined as the measured concentration of Aβ40 in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of Aβ40 in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 CSF samples	Buffer Corrected Grand mean: 94.9% Range: 86.7% - 106%
Spike Concentrations	Control: Calibrator Diluent Low: 5,000 pg/mL High: 17,500 pg/mL	
Replicates/Run (each test sample)	3	

**Dilution Linearity (Plasma):**

2 normal human plasma samples spiked with recombinant analyte and 2 unspiked normal human plasma samples were diluted 2x serially with Sample diluent from MRD (4x) to 256x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human Plasma 2 Spiked Normal Human Plasma	Endogenous Plasma Dilution Linearity (8x total dilution): 112% Range: 103-125%  Spiked Plasma Dilution Linearity (32x total dilution) 101% Range: 89.3-115%
Dilutions	2X serial from MRD (4X)	
Replicates/Run (each test sample)	3	

**Dilution Linearity (CSF):**

2 normal human CSF samples spiked with recombinant analyte and 2 unspiked normal human CSF samples were diluted 2x serially with Sample diluent from MRD (400x) to 25600x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human CSF 2 Spiked Normal Human CSF	Endogenous CSF Dilution Linearity (800x total dilution): 92% Range: 87.9-97.2%  Spiked CSF Dilution Linearity (12800x total dilution) 88% Range: 78.8-101%
Dilutions	2X serial from MRD (400x)	
Replicates/Run (each test sample)	3	

**Curve Storage:**

3 Panels and 2 controls were diluted 1:4 in sample diluent. 4 runs were run on 3 individual days on the same instrument using one lot of reagents. The mean total bias is determined by the concentration bias for each control and panel derived from each of other 3 calibration curves relative to the concentration determined from the native curve.

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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator	Mean Total Bias: 1.22%
Specifications	Replicates/Sample: 3 Instruments: 1 Runs/Instrument: 4 Lots Tested: 1 Total Runs: 4	

**Drift:**

2 Panels and 2 controls were diluted 1:4 in sample diluent. Controls and panels were run across three plates with a total of 54 replicates per sample. The Plate 1, Plate 1+2 and Plate 1+2+3 variance values are determined by the absolute bias of end job/beginning job for each sample at each period. The three-plate precision is determined by the within run CV for each sample.

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 Plasma Sample spiked with kit calibrator Panel 2: Normal Human Plasma Sample spiked with kit calibrator	Three-plate Variance: Plate 1: 7.5% Plate 1+2: 5.0% Plate 1+2+3: 4.9%
Specifications	Replicates/Sample: 3 Replicates of Sample/Plate: 6 Plates: 3 Total Replicates: 54	Three-plate Precision: 3.5% Range: 2.6 – 4.5%

**Normal Sample Reading (Plasma):**

Plasma samples were collected from 20 healthy individuals. Plasma samples were diluted 1:4 with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	20 Normal Human Plasma	Mean Plasma: 90.9 pg/mL Plasma Range: 69.1 – 112 pg/mL Detectability: 100% Quantifiability: 100%
Replicates (each test sample)	3	

**Normal Sample Reading (CSF):**

CSF samples were collected from 25 healthy individuals. CSF samples were diluted 1:400 off board with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	25 Normal Human CSF	Mean CSF: 4862 pg/mL CSF Range: 79.7 – 13899 pg/mL Detectability: 96% Quantifiability: 96%
Replicates (each test sample)	3	

**III. Aβ42 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: 0.136 pg/mL  Range: 0.0601 - 0.204 pg/mL
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Limit of Quantification:**

The table below shows the calculated LLOQ (Lower Limit of Quantification). Analytical LLOQ is the lowest concentration of calibrator with ≤ 20% pooled CV and 80 – 120% mean recovery over all runs. Functional LLOQ is the Analytical LLOQ multiplied by the Minimum Required Dilution.

Test Conditions		Claim
Samples	Calibrator B at 0.378 pg/mL and diluted to 0.189 pg/mL Calibrator C at 0.780 pg/mL and diluted to 0.390 pg/mL and 0.195 pg/mL	Analytical LLOQ: 0.378 pg/mL  Functional LLOQ (EDTA Plasma) (4x) 1.51 pg/mL  Functional LLOQ (CSF) (400x) 151 pg/mL
Replicates/Run (each test sample)	3	
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Precision Profile:**

3 Plasma Panels and 2 controls were diluted 1:4 in Plasma Sample Diluent and 1 CSF Panel was diluted 1:400 in CSF Sample Diluent. 12 runs were run on 2 instruments comparing 2 reagent lots. The mean concentration corrected for MRD (pg/mL) and % CV 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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator Panel 4: Normal Human CSF Sample	Mean Total CV: 3.1%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 3 Lots Tested: 2 Total Runs: 12; Total Measurements/Sample: 36	
Within Run CV		3.4%
Between Run CV		5.5%
Between Instrument CV		1.0 %
Between Lot CV		2.5%

**Spike Recovery (Plasma):**

2 normal plasma samples and a Diluent Control were spiked at two concentrations (high and low) with Aβ42 antigen and then diluted 1:4 with Sample Diluent. Percent recovery is defined as the measured concentration of Aβ42 in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of Aβ42 in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 EDTA plasma	Buffer Corrected Grand mean: 71.7 % Range: 65.4% - 75.7%
Spike Concentrations	Control: Calibrator Diluent Low: 40 pg/mL High: 80 pg/mL	
Replicates/Run (each test sample)	3	

**Spike Recovery (CSF):**

2 normal CSF samples and a Diluent Control were diluted 1:400 in Sample diluent and then spiked at two concentrations (high and low) with Aβ42 antigen. Percent recovery is defined as the measured concentration of Aβ42 in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of Aβ42 in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 CSF samples	Buffer Corrected Grand mean: 90.8% Range: 85.4% - 95.0%
Spike Concentrations	Control: Calibrator Diluent Low: 4,000 pg/mL High: 8,000 pg/mL	
Replicates/Run (each test sample)	3	

**Dilution Linearity (Plasma):**

2 normal human plasma samples spiked with recombinant analyte and 2 unspiked normal human plasma samples were diluted 2x serially with Sample diluent from MRD (4x) to 256x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human EDTA Plasma 2 Spiked Normal Human Plasma	Endogenous Plasma Dilution Linearity (8x total dilution): 93% Range: 85.6- 100%
Dilutions	2X serial from MRD (4X)	
Replicates/Run (each test sample)	3	Spiked Plasma Dilution Linearity (32x total dilution) 101% Range: 90.1- 123%

**Dilution Linearity (CSF):**

2 normal human CSF samples spiked with recombinant analyte and 2 unspiked normal human CSF samples were diluted 2x serially with Sample diluent from MRD (400x) to 25600x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human CSF 2 Spiked Normal Human CSF	Endogenous CSF Dilution Linearity (800x total dilution): 99% Range: 96.1- 101%
Dilutions	2X serial from MRD (400x)	
Replicates/Run (each test sample)	3	Spiked CSF Dilution Linearity (12800x total dilution) 107% Range: 89.7-158%

**Curve Storage:**

3 Panels and 2 controls were diluted 1:4 in sample diluent. 4 runs were run on 3 individual days on the same instrument using one lot of reagents. The mean total bias is determined by the concentration bias for each control and panel derived from each of other 3 calibration curves relative to the concentration determined from the native curve.

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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator	Mean Total Bias: 0.91%
Specifications	Replicates/Sample: 3 Instruments: 1 Runs/Instrument: 4 Lots Tested: 1 Total Runs: 4	

**Drift:**

2 Panels and 2 controls were diluted 1:4 in sample diluent. Controls, panels 1 and 2 were run across three plates with a total of 54 replicates per sample. The Plate 1, Plate 1+2 and Plate 1+2+3 variance values are determined by the absolute bias of end job/beginning job for each sample at each period. The three-plate precision is determined by the within run CV for each sample.

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 Plasma Sample spiked with kit calibrator Panel 2: Normal Human Plasma Sample spiked with kit calibrator	Three-plate Variance: Plate 1: 8.7% Plate 1+2: 4.6% Plate 1+2+3: 5.8%
Specifications	Replicates/Sample: 3 Replicates of Sample/Plate: 6 Plates: 3 Total Replicates: 54	Three-plate Precision: 3.8% Range: 2.4–4.8 %

**Normal Sample Reading (Plasma):**

Plasma samples were collected from 20 healthy individuals. Plasma samples were diluted 1:4 with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	20 Normal Human Plasma	Mean Plasma: 5.87pg/mL Plasma Range: 4.49–7.85 pg/mL Detectability: 100% Quantifiability: 100%
Replicates (each test sample)	3	

**Normal Sample Reading (CSF):**

CSF samples were collected from 25 healthy individuals. CSF samples were diluted 1:400 off board with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	25 Normal Human CSF	Mean CSF: 321 pg/mL CSF Range: 79.6–1025 pg/mL Detectability: 100% Quantifiability: 80%
Replicates (each test sample)	3	

**IV. 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: 0.441 pg/mL  Range: 0.240 - 0.757 pg/mL
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	11*	

\*One run contained LOD readback below Cal A

**Limit of Quantification:**

The table below shows the calculated LLOQ (Lower Limit of Quantification). Analytical LLOQ is the lowest concentration of calibrator with ≤ 20% pooled CV and 80 – 120% mean recovery over all runs. Functional LLOQ is the Analytical LLOQ multiplied by the Minimum Required Dilution.

Test Conditions		Claim
Samples	Calibrator B at 5.78 pg/mL and diluted to 2.89 pg/mL Calibrator C at 17.2 pg/mL and diluted to 8.62 pg/mL and 4.31 pg/mL	Analytical LLOQ: 2.89 pg/mL  Functional LLOQ (EDTA Plasma) (4x) 11.6 pg/mL  Functional LLOQ (CSF) (400x) 1156 pg/mL
Replicates/Run (each test sample)	3	
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Precision Profile:**

3 Plasma Panels and 2 controls were diluted 1:4 in Plasma Sample Diluent and 1 CSF Panel was diluted 1:400 in CSF Sample Diluent. 12 runs were run on 2 instruments comparing 2 reagent lots. The mean concentration corrected for MRD (pg/mL) and % CV 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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator Panel 4: Normal Human CSF Sample	Mean Total CV: 5.0%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 3 Lots Tested: 2 Total Runs: 12; Total Measurements/Sample: 36	
Within Run CV		5.1%
Between Run CV		8.6%
Between Instrument CV		2.2 %
Between Lot CV		4.3%

**Spike Recovery (Plasma):**

2 normal plasma samples and a Diluent Control were spiked at two concentrations (high and low) with GFAP antigen and then diluted 1:4 with Sample Diluent. Percent recovery is defined as the measured concentration of GFAP in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of GFAP in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 EDTA plasma	Buffer Corrected Grand mean: 58.1% Range: 49.5% - 64.8%
Spike Concentrations	Control: Calibrator Diluent Low: 600 pg/mL High: 3,000 pg/mL	
Replicates/Run (each test sample)	3	

**Spike Recovery (CSF):**

2 normal CSF samples and a Diluent Control were diluted 1:400 in Sample diluent and then spiked at two concentrations (high and low) with GFAP antigen. Percent recovery is defined as the measured concentration of GFAP in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of GFAP in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 CSF samples	Buffer Corrected Grand mean: 91.4% Range: 84.4% - 96.7%
Spike Concentrations	Control: Calibrator Diluent Low 60,000 pg/mL High: 300,000 pg/mL	
Replicates/Run (each test sample)	3	

**Dilution Linearity (Plasma):**

2 normal human plasma samples spiked with recombinant analyte and 2 unspiked normal human plasma samples were diluted 2x serially with Sample diluent from MRD (4x) to 256x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human EDTA Plasma 2 Spiked Normal Human EDTA Plasma	Endogenous Plasma Dilution Linearity (8x total dilution): 117% Range: 108-129%
Dilutions	2X serial from MRD (4X)	Spiked Plasma Dilution Linearity (32x total dilution) 118% Range: 105 -140 %
Replicates/Run (each test sample)	3	

**Dilution Linearity (CSF):**

2 normal human CSF samples spiked with recombinant analyte and 2 unspiked normal human CSF samples were diluted 2x serially with Sample diluent from MRD (400x) to 25600x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human CSF 2 Spiked Normal Human CSF	Endogenous CSF Dilution Linearity (800x total dilution): 102% Range: 99.9-104%
Dilutions	2X serial from MRD(400x)	Spiked CSF Dilution Linearity (12800x total dilution) 93% Range: 76.9- 102%
Replicates/Run (each test sample)	3	

**Curve Storage:**

3 Panels and 2 controls were diluted 1:4 in sample diluent. 4 runs were run on 3 individual days on the same instrument using one lot of reagents. The mean total bias is determined by the concentration bias for each control and panel derived from each of other 3 calibration curves relative to the concentration determined from the native curve.

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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator	Mean Total Bias: 6.38%
Specifications	Replicates/Sample: 3 Instruments: 1 Runs/Instrument: 4 Lots Tested: 1 Total Runs: 4	

**Drift:**

2 Panels and 2 controls were diluted 1:4 in sample diluent. Controls, panels 1 and 2 were run across three plates with a total of 54 replicates per sample. The Plate 1, Plate 1+2 and Plate 1+2+3 variance values are determined by the absolute bias of end job/beginning job for each sample at each period. The three-plate precision is determined by the within run CV for each sample.

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 Plasma Sample spiked with kit calibrator Panel 2: Normal Human Plasma Sample spiked with kit calibrator	Three-plate Variance: Plate 1: 12.4% Plate 1+2: 9.6% Plate 1+2+3: 5.9%  Three-plate Precision: 7.9% Range: 4.2– 8.7%
Specifications	Replicates/Sample: 3 Replicates of Sample/Plate: 6 Plates: 3 Total Replicates: 54	

**Normal Sample Reading (Plasma):**

Plasma samples were collected from 20 healthy individuals. Plasma samples were diluted 1:4 with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	20 Normal Human Plasma	Mean Plasma: 58.8 pg/mL Plasma Range: 19.9–181 pg/mL Detectability: 100% Quantifiability: 100%
Replicates (each test sample)	3	

**Normal Sample Reading (CSF):**

CSF samples were collected from 25 healthy individuals. CSF samples were diluted 1:400 off board with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	25 Normal Human CSF	Mean CSF: 17426 pg/mL CSF Range: 478–94189 pg/mL Detectability: 100% Quantifiability: 92%
Replicates (each test sample)	3	

**v. NF-L 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: 0.090 pg/mL  Range: 0.016 to 0.152 pg/mL
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Limit of Quantification:**

The table below shows the calculated LLOQ (Lower Limit of Quantification). Analytical LLOQ is the lowest concentration of calibrator with ≤ 20% pooled CV and 80 – 120% mean recovery over all runs. Functional LLOQ is the Analytical LLOQ multiplied by the Minimum Required Dilution.

Test Conditions		Claim
Samples	Calibrator B at 0.519 pg/mL and diluted to 0.260 pg/mL Calibrator C at 1.60 pg/mL and diluted to 0.801 pg/mL and 0.400 pg/mL	Analytical LLOQ: 0.400 pg/mL  Functional LLOQ (EDTA Plasma) (4x) 1.60 pg/mL  Functional LLOQ (CSF) (400x) 160 pg/mL
Replicates/Run (each test sample)	3	
# Instruments	2	
Runs/Instrument	3	
# Lots Tested	2	
Total Runs	12	

**Precision Profile:**

3 Plasma Panels and 2 controls were diluted 1:4 in Plasma Sample Diluent and 1 CSF Panel was diluted 1:400 in CSF Sample Diluent. 12 runs were run on 2 instruments comparing 2 reagent lots. The mean concentration corrected for MRD (pg/mL) and % CV 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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator Panel 4: Normal Human CSF Sample	Mean Total CV: 5.1%
Specifications	Replicates/Sample: 3 Instruments: 2 Runs/Instrument: 3 Lots Tested: 2 Total Runs: 12; Total Measurements/Sample: 36	
Within Run CV		6.0%
Between Run CV		7.3%
Between Instrument CV		2.5 %
Between Lot CV		4.4%

**Spike Recovery (Plasma):**

2 normal plasma samples and a Diluent Control were spiked at two concentrations (high and low) with NF-L antigen and then diluted 1:4 with Sample Diluent. Percent recovery is defined as the measured concentration of NF-L in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of NF-L in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 EDTA plasma	Buffer Corrected Grand mean: 111% Range: 94.9% -120%
Spike Concentrations	Control: Calibrator Diluent Low: 150 pg/mL High: 750 pg/mL	
Replicates/Run (each test sample)	3	

**Spike Recovery (CSF):**

2 normal CSF samples and a Diluent Control were diluted 1:400 in Sample diluent and then spiked at two concentrations (high and low) with NF-L antigen. Percent recovery is defined as the measured concentration of NF-L in the spiked sample less than the measured concentration in unspiked sample relative to the concentration of NF-L in spiked calibrator diluent.

Test Conditions		Claim
Test Samples	Normal Human Samples – 2 CSF samples	Buffer Corrected Grand mean: 88.7% Range: 80.6% -93.4%
Spike Concentrations	Control: Calibrator Diluent Low: 15,000 pg/mL High: 75,000 pg/mL	
Replicates/Run (each test sample)	3	

**Dilution Linearity (Plasma):**

2 normal human plasma samples spiked with recombinant analyte and 2 unspiked normal human plasma samples were diluted 2x serially with Sample diluent from MRD (4x) to 256x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human Plasma 2 Spiked Normal Human Plasma	Endogenous Plasma Dilution Linearity (8x total dilution): 111% Range: 91.2 - 118%
Dilutions	2X serial from MRD (4X)	
Replicates/Run (each test sample)	3	Spiked Plasma Dilution Linearity (32x total dilution) 97% Range: 86 – 104%

**Dilution Linearity (CSF):**

2 normal human CSF samples spiked with recombinant analyte and 2 unspiked normal human CSF samples were diluted 2x serially with Sample diluent from MRD (400x) to 25600x. The average percent recovery across the valid (>LLOQ) dilution series is displayed for each sample.

Test Conditions		Claim
Test Samples	2 Endogenous Normal Human CSF 2 Spiked Normal Human CSF	Endogenous CSF Dilution Linearity (800x total dilution): 91% Range: 86.6 - 96%
Dilutions	2X serial from MRD(400x)	
Replicates/Run (each test sample)	3	Spiked CSF Dilution Linearity (12800x total dilution) 92% Range: 83.3 - 100 %

**Curve Storage:**

3 Panels and 2 controls were diluted 1:4 in sample diluent. 4 runs were run on 3 individual days on the same instrument using one lot of reagents. The mean total bias is determined by the concentration bias for each control and panel derived from each of other 3 calibration curves relative to the concentration determined from the native curve.

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 Plasma Sample Panel 2: Normal Human Plasma Sample spiked with kit calibrator Panel 3: Normal Human Plasma Sample spiked with kit calibrator	Mean Total Bias: 2.36%
Specifications	Replicates/Sample: 3 Instruments: 1 Runs/Instrument: 4 Lots Tested: 1 Total Runs: 4	

**Drift:**

2 Panels and 2 controls were diluted 1:4 in sample diluent. Controls, panels 1 and 2 were run across three plates with a total of 54 replicates per sample. The Plate 1, Plate 1+2 and Plate 1+2+3 variance values are determined by the absolute bias of end job/beginning job for each sample at each period. The three-plate precision is determined by the within run CV for each sample.

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 Plasma Sample spiked with kit calibrator Panel 2: Normal Human Plasma Sample spiked with kit calibrator	Three-plate Variance: Plate 1: 13.6% Plate 1+2: 11.1% Plate 1+2+3: 11.2%
Specifications	Replicates/Sample: 3 Replicates of Sample/Plate: 6 Plates: 3 Total Replicates: 54	Three-plate Precision: 8.58% Range: 4.5– 9.1%

**Normal Sample Reading (Plasma):**

Plasma samples were collected from 20 healthy individuals. Plasma samples were diluted 1:4 with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	20 Normal Human Plasma	Mean Plasma: 13.6 pg/mL Plasma Range: 2.80– 49.0 pg/mL Detectability: 100% Quantifiability: 100%
Replicates (each test sample)	3	

**Normal Sample Reading (CSF):**

CSF samples were collected from 25 healthy individuals. CSF samples were diluted 1:400 off board with Sample Diluent and tested in triplicate. The mean concentrations in pg/mL for each sample are displayed in the table below.

Test Conditions		Claim
Test Samples	25 Normal Human CSF	Mean CSF: 2743 pg/mL CSF Range: 249–25722 pg/mL Detectability: 100% Quantifiability: 100%
Replicates (each test sample)	3	

## VI. Aβ40 Claims Detail

### LOD Summary

Neurology 4-Plex E Advantage Kit Assay Characterization		
Analytical LOQ	1.02 pg/mL	n=12, Derived from primary and reference calibration curves with additional 1:2 and 1:4 dilution of CalC and 1:2 dilution of CalB, 2 lots testing across 2 instruments per lot, 12 runs total
Functional LOQ	EDTA Plasma (4x) 4.08 pg/mL CSF (400x) 408 pg/mL	
LOD	0.384 ± 0.114 pg/mL (0.189 – 0.531 pg/mL)	n=12, Grand mean of 2.5 SD LOD calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
B/A	4.14 ± 0.772 Range: 3.18-5.54	n=12, Grand mean of B/A calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal A	0.004 ± 0.001 Range: 0.003-0.005	n=12, Grand mean of Cal A from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal H	11.4 ± 0.860 Range: 10.5-13.0	n=12, Grand mean of Cal H from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total

### LOD Lot 1

Reagent Lot	Lot 1							
Instrument	HDX-54			HDX-07			AVE	SD
Primary Cal LOD pg/mL	0.308	0.437	0.459	0.476	0.531	0.305	0.419	0.093
Primary Cal B/A	3.5	3.3	3.6	3.6	4.0	5.4	3.91	0.749
Primary Cal A	0.005	0.005	0.004	0.004	0.004	0.003	0.004	0.001
Primary Cal H	13.0	12.1	12.7	11.8	11.6	11.4	12.1	0.618

### LOD Lot 2

Reagent Lot	Lot 2							
Instrument	HDX-07			HDX-54			AVE	SD
Reference Cal LOD pg/mL	0.189	0.507	0.419	0.462	0.257	0.255	0.348	0.131
Reference Cal B/A	4.4	5.5	3.2	4.6	3.9	4.6	4.37	0.788
Reference Cal A	0.004	0.003	0.005	0.004	0.004	0.004	0.004	0.0004
Reference Cal H	10.6	10.6	10.7	10.9	10.9	10.5	10.7	0.185

**LLOQ Lot 1**

% Recovery						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
2.03 pg/mL	2%	4%	5%	5%	6%	5%
1.13 pg/mL	10%	1%	7%	5%	5%	6%
1.02 pg/mL	7%	12%	11%	10%	1%	4%
0.563 pg/mL	18%	7%	11%	11%	20%	13%
0.508 pg/mL	14%	19%	15%	6%	8%	16%
Pooled CV						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
2.03 pg/mL	101%	102%	99%	99%	98%	101%
1.13 pg/mL	106%	106%	111%	108%	113%	114%
1.02 pg/mL	108%	110%	113%	93%	114%	121%
0.563 pg/mL	117%	117%	132%	112%	121%	119%
0.508 pg/mL	136%	128%	126%	124%	117%	163%

**LLOQ Lot 2**

% Recovery						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
2.03 pg/mL	7%	1%	4%	3%	2%	3%
1.13 pg/mL	10%	4%	7%	1%	15%	4%
1.02 pg/mL	9%	6%	1%	7%	10%	6%
0.563 pg/mL	9%	1%	10%	20%	6%	10%
0.508 pg/mL	12%	4%	14%	13%	19%	9%
Pooled CV						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
2.03 pg/mL	101%	101%	106%	99%	102%	102%
1.13 pg/mL	101%	103%	92%	105%	94%	100%
1.02 pg/mL	110%	106%	120%	109%	111%	110%
0.563 pg/mL	133%	133%	100%	123%	138%	111%
0.508 pg/mL	119%	141%	137%	117%	130%	136%

**LLOQ Summary**

LOQ Point	LOQ Point (pg/mL)	Pooled CV	Mean Recovery	LLOQ Claim (pg/mL)
High	2.03	4.5%	101%	<b>1.02</b>
Mid-High	1.13	7.5%	104%	
Mid	1.02	8.0%	110%	
Low-Mid	0.563	14.0%	121%	
Low	0.508	13.2%	131%	

**Precision Sample Results**

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	14.6	2.0%	2.3%	0.1%	0.25%
Control 2	107	3.4%	2.4%	0.49%	1.61%
Panel 1	14.7	3.1%	2.6%	1.0%	1.2%
Panel 2	52.0	1.6%	3.1%	0.2%	1.1%
Panel 3	127	2.6%	3.8%	0.8%	2.4%
Panel 4	6848	2.0%	4.6%	0.7%	3.5%

**Spike Recovery (Plasma)**

Aβ40 (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	50 pg/mL	175 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	70%	70%	70%	68%
CSF 2	70%	62%	66%	

**Spike Recovery (CSF)**

Aβ40 (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	5,000 pg/mL	17,500 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	104%	88%	96%	95%
CSF 2	99%	89%	94%	

**Dilution Linearity (Plasma)**

Dilution	Endogenous Plasma	Spiked Plasma
MRD (4x)	100%	100%
2x MRD	112%	104%
4x MRD	*	100%
8x MRD	*	100%

\*Values below LLOQ

**Dilution Linearity (CSF)**

Dilution	Endogenous CSF	Spiked CSF
MRD (4x)	100%	100%
2x MRD	92%	98%
4x MRD	*	91%
8x MRD	*	88%
16x MRD	*	82%
32x MRD	*	83%

\*Values below LLOQ

**Curve Storage**

%Bias	Run 1 Curve	Run 2 Curve	Run 3 Curve	Run 4 Curve
Run 1	0.0%	2.2%	1.4%	0.7%
Run 2	2.3%	0.0%	0.8%	1.5%
Run 3	1.5%	0.7%	0.0%	0.7%
Run 4	0.8%	1.4%	0.7%	0.0%

**Drift**

Sample	Plate 1 End/Start	Plate 1+2 End/Start	Plate 1+2+3 End/Start	Within Run CV
Control 1	0.4%	4.2%	4.7%	2.8%
Control 2	17.8%	9.5%	6.5%	4.5%
Panel 1	0.8%	1.7%	0.1%	2.6%
Panel 2	10.8%	4.6%	8.4%	4.2%

**Normal Sample Reading (Human)**

Aβ40	Human Samples			
	EDTA Plasma conc (pg/mL)	CV	CSF conc (pg/mL)	CV
1	80.2	3.1%	79.7	---
2	69.1	4.2%	3915	2.3%
3	87.9	1.5%	6465	0.6%
4	70.7	1.4%	6327	1.8%
5	98.9	2.0%	10489	2.9%
6	98.7	0.6%	5395	1.8%
7	83.6	1.2%	4582	0.6%
8	90.9	1.5%	3010	2.2%
9	112	0.6%	3181	1.2%
10	101	4.8%	6280	1.4%
11	111	3.1%	4104	2.3%
12	71.0	2.8%	3959	2.0%
13	108	0.6%	2333	1.0%
14	82.4	2.7%	4982	1.1%
15	110	3.1%	2267	1.7%
16	74.8	3.3%	13899	1.1%
17	98.7	3.3%	2723	3.1%
18	86.3	1.6%	995	3.1%
19	107	2.6%	2453	1.1%
20	76.1	3.9%	2843	0.3%
21	---	---	4337	2.0%
22	---	---	10445	3.8%
23	---	---	4546	2.6%
24	---	---	3953	1.0%
25	---	---	3215	2.0%
<b>Mean</b>	<b>90.9 pg/ml</b>		<b>4862 pg/mL</b>	
<b>Median</b>	<b>89.4 pg/ml</b>		<b>3959 pg/mL</b>	
<b>Range</b>	<b>69.1 – 112 pg/ml</b>		<b>79.7- 13899</b>	
<b>Detectability</b>	100%		96%	
<b>Quantifiability</b>	100%		96%	

## VII. Aβ42 Claims Detail

### LOD Summary

Neurology 4-Plex E Advantage Kit Assay Characterization		
Analytical LOQ	0.378 pg/mL	n=12, Derived from primary and reference calibration curves with additional 1:2 and 1:4 dilution of CalC and 1:2 dilution of CalB, 2 lots testing across 2 instruments per lot, 12 runs total
Functional LOQ	EDTA Plasma (4x): 1.51 pg/mL CSF (400x): 151 pg/mL	
LOD	0.136 ± 0.054 (0.0601 -0.204 pg/mL)	n=12, Grand mean of 2.5 SD LOD calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
B/A	3.25 ± 0.490 Range: 2.60-4.07	n=12, Grand mean of B/A calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal A	0.004 ± 0.001 Range: 0.003-0.004	n=12, Grand mean of Cal A from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal H	13.0 ± 0.824 Range: 11.9-14.5	n=12, Grand mean of Cal H from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total

### LOD Lot 1

Reagent Lot	Lot 1							
Instrument	HDX-54			HDX-07			AVE	SD
Primary Cal LOD pg/mL	0.197	0.171	0.175	0.120	0.204	0.204	0.179	0.032
Primary Cal B/A	2.6	2.6	2.8	3.4	4.1	4.1	3.26	0.688
Primary Cal A	0.004	0.004	0.004	0.003	0.003	0.003	0.004	0.001
Primary Cal H	14.5	13.8	14.4	13.2	12.9	12.6	13.6	0.807

### LOD Lot 2

Reagent Lot	Lot 2							
Instrument	HDX-07			HDX-54			AVE	SD
Reference Cal LOD pg/mL	0.135	0.089	0.130	0.061	0.060	0.089	0.094	0.032
Reference Cal B/A	3.2	3.3	2.9	3.0	3.5	3.5	3.24	0.233
Reference Cal A	0.004	0.004	0.004	0.004	0.004	0.003	0.004	0.000
Reference Cal H	12.4	12.2	12.9	12.9	12.6	11.9	12.5	0.403

**LLOQ Lot 1**

% Recovery						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
0.780 pg/mL	6%	8%	3%	0%	5%	6%
0.390 pg/mL	6%	11%	9%	6%	6%	6%
0.378 pg/mL	2%	12%	5%	9%	3%	3%
0.195 pg/mL	16%	16%	27%	21%	20%	25%
0.189 pg/mL	22%	10%	6%	25%	5%	25%
Pooled CV						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
0.780 pg/mL	96%	97%	96%	94%	94%	94%
0.390 pg/mL	99%	97%	103%	108%	109%	106%
0.378 pg/mL	103%	35%	37%	39%	40%	35%
0.195 pg/mL	102%	46%	35%	39%	45%	42%
0.189 pg/mL	140%	50%	40%	35%	45%	43%

**LLOQ Lot 2**

% Recovery						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
0.780 pg/mL	1%	5%	2%	3%	3%	4%
0.390 pg/mL	8%	7%	5%	7%	12%	5%
0.378 pg/mL	5%	4%	5%	8%	4%	8%
0.195 pg/mL	7%	25%	13%	60%	21%	7%
0.189 pg/mL	29%	4%	3%	9%	21%	12%
Pooled CV						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
0.780 pg/mL	100%	100%	98%	105%	99%	101%
0.390 pg/mL	100%	101%	102%	97%	102%	97%
0.378 pg/mL	34%	36%	39%	37%	40%	64%
0.195 pg/mL	41%	37%	40%	31%	47%	65%
0.189 pg/mL	30%	36%	31%	31%	29%	44%

**LLOQ Summary**

LOQ Point	LOQ Point (pg/mL)	Pooled CV	Mean Recovery	LLOQ Claim (pg/mL)
High	0.780	4.7%	97.7%	<b>0.378</b>
Mid-High	0.390	8.0%	45.0%	
Mid	0.378	6.6%	102%	
Low-Mid	0.195	26.2%	47.6%	
Low	0.189	17.7%	46.0%	

**Precision Sample Results**

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	5.43	2.3%	2.9%	1.5%	0.72%
Control 2	29.0	3.1%	3.3%	0.01%	2.87%
Panel 1	1.37	8.7%	11.0%	2.9%	8.2%
Panel 2	20.3	1.4%	4.2%	0.1%	1.3%
Panel 3	42.6	2.5%	3.3%	0.3%	0.7%
Panel 4	468	2.7%	8.2%	1.3%	0.9%

**Spike Recovery (Plasma)**

<b>Aβ42 (w/ Buffer Correction) % Recovery</b> <b>[(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100</b>				
Sample	40 pg/mL	80 pg/mL	Mean	Grand Mean
Diluent	99%	100%	-	-
CSF 1	75%	75%	75%	72%
CSF 2	70%	66%	68%	

**Spike Recovery (CSF)**

<b>Aβ42 (w/ Buffer Correction) % Recovery</b> <b>[(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100</b>				
Sample	4,000 pg/mL	8,000 pg/mL	Mean	Grand Mean
Diluent	99%	99%	-	-
CSF 1	93%	90%	92%	91%
CSF 2	90%	90%	90%	

**Dilution Linearity (Plasma)**

Dilution	Endogenous Plasma	Spiked Plasma
MRD (4x)	100%	100%
2x MRD	93%	93%
4x MRD	*	102%
8x MRD	*	109%

\*Values below LLOQ

**Dilution Linearity (CSF)**

Dilution	Endogenous CSF	Spiked CS
MRD (4x)	100%	100%
2x MRD	99%	105%
4x MRD	*	103%
8x MRD	*	106%
16x MRD	*	102%
32x MRD	*	119%

\*Values below LLOQ

**Curve Storage**

%Bias	Run 1 Curve	Run 2 Curve	Run 3 Curve	Run 4 Curve
Run 1	0.0%	0.7%	1.9%	0.0%
Run 2	1.1%	0.0%	0.7%	0.7%
Run 3	1.7%	0.5%	0.0%	1.2%
Run 4	0.5%	0.7%	1.3%	0.0%

**Drift**

Sample	Plate 1 End/Start	Plate 1+2 End/Start	Plate 1+2+3 End/Start	Within Run CV
Control 1	2.3%	1.1%	1.6%	3.2%
Control 2	17.6%	10.7%	7.3%	4.8%
Panel 1	2.2%	0.5%	2.9%	2.4%
Panel 2	12.6%	6.0%	11.4%	4.8%

**Normal Sample Reading (Human)**

Aβ42	Human Samples			
	EDTA Plasma conc (pg/mL)	CV	CSF conc (pg/mL)	CV
1	6.06	3.9%	94	28%
2	4.49	6.4%	288	8%
3	5.02	2.9%	474	5%
4	5.11	1.1%	224	5%
5	6.16	3.3%	323	8%
6	7.09	0.8%	310	4%
7	5.62	2.5%	369	5%
8	5.76	4.2%	201	2%
9	7.85	1.9%	237	10%
10	6.41	5.7%	484	3%
11	7.39	2.7%	287	7%
12	4.96	1.6%	253	3%
13	6.94	0.2%	91	22%
14	6.44	3.0%	252	4%
15	6.82	4.5%	206	2%
16	4.85	1.8%	1025	2%
17	5.36	6.6%	142	10%
18	5.36	5.9%	80	28%
19	4.81	2.4%	158	13%
20	4.90	4.8%	170	6%
21	---	---	250	1%
22	---	---	428	3%
23	---	---	234	5%
24	---	---	258	5%
25	---	---	148	13%
<b>Mean</b>	<b>5.87 pg/ml</b>		<b>321 pg/mL</b>	
<b>Median</b>	<b>5.69 pg/ml</b>		<b>250 pg/mL</b>	
<b>Range</b>	<b>4.49-7.85 pg/ml</b>		<b>79.6-1025 pg/mL</b>	
<b>Detectability</b>	100%		96%	
<b>Quantifiability</b>	100%		80%	

**VIII. GFAP Claims Detail**

**LOD Summary**

Neurology 4-Plex E Advantage Kit Assay Characterization		
Analytical LOQ	2.89 pg/mL	n=12, Derived from primary and reference calibration curves with additional 1:2 and 1:4 dilution of CalC and 1:2 dilution of CalB, 2 lots testing across 2 instruments per lot, 12 runs total
Functional LOQ	EDTA Plasma (4x): 11.6 pg/mL CSF (400x): 1156 pg/mL	
LOD	0.441 ± 0.179 (0.240 – 0.757 pg/mL)	n=12, Grand mean of 2.5 SD LOD calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
B/A	7.66 ± 0.687 Range: 6.43-9.13	n=12, Grand mean of B/A calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal A	0.003 ± 0.0003 Range: 0.003-0.004	n=12, Grand mean of Cal A from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal H	14.2 ± 1.205 Range: 12.5-16.3	n=12, Grand mean of Cal H from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total

**LOD Lot 1**

Reagent Lot	Lot 1							
Instrument	HDX-54			HDX-07			AVE	SD
Primary Cal LOD pg/mL	0.576	NAN	0.279	0.717	0.467	0.757	0.559	0.194
Primary Cal B/A	6.4	7.5	7.3	7.2	8.1	7.3	7.30	0.535
Primary Cal A	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000
Primary Cal H	13.0	12.5	13.1	13.3	13.5	13.2	13.1	0.336

**LOD Lot 2**

Reagent Lot	Lot 2							
Instrument	HDX-07			HDX-54			AVE	SD
Reference Cal LOD pg/mL	0.482	0.324	0.308	0.240	0.414	0.287	0.343	0.089
Reference Cal B/A	8.1	9.1	8.1	7.5	7.2	8.2	8.03	0.659
Reference Cal A	0.004	0.003	0.003	0.004	0.004	0.003	0.003	0.0003
Reference Cal H	14.9	15.3	16.3	15.0	15.2	14.8	15.3	0.566

**LLOQ Lot 1**

% Recovery						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
17.2 pg/mL	10%	4%	10%	9%	8%	10%
8.62 pg/mL	7%	21%	8%	9%	11%	15%
5.78 pg/mL	3%	7%	6%	11%	11%	6%
4.31 pg/mL	10%	12%	15%	4%	1%	5%
2.89 pg/mL	32%	7%	11%	25%	6%	12%
Pooled CV						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
17.2 pg/mL	102%	99%	104%	96%	102%	100%
8.62 pg/mL	101%	109%	101%	106%	103%	101%
5.78 pg/mL	110%	80%	87%	95%	88%	99%
4.31 pg/mL	92%	82%	76%	81%	79%	117%
2.89 pg/mL	88%	89%	70%	81%	86%	100%

**LLOQ Lot 2**

% Recovery						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
17.2 pg/mL	5%	5%	4%	5%	5%	1%
8.62 pg/mL	6%	9%	8%	2%	8%	5%
5.78 pg/mL	6%	5%	1%	19%	1%	3%
4.31 pg/mL	3%	3%	4%	10%	10%	8%
2.89 pg/mL	6%	3%	7%	8%	5%	6%
Pooled CV						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
17.2 pg/mL	101%	105%	99%	99%	104%	102%
8.62 pg/mL	104%	97%	103%	106%	100%	100%
5.78 pg/mL	89%	96%	89%	103%	90%	97%
4.31 pg/mL	91%	84%	90%	91%	78%	94%
2.89 pg/mL	96%	95%	100%	86%	99%	97%

**LLOQ Summary**

LOQ Point	LOQ Point (pg/mL)	Pooled CV	Mean Recovery	LLOQ Claim (pg/mL)
High	17.2	7.2%	101%	<b>2.89</b>
Mid-High	8.62	8.6%	93.6%	
Mid	5.78	10.6%	103%	
Low-Mid	4.31	8.5%	87.9%	
Low	2.89	14.2%	90.7%	

**Precision Sample Results**

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	215	3.0%	5.0%	2.2%	4.91%
Control 2	4358	5.8%	4.2%	2.48%	2.03%
Panel 1	26.7	9.3%	14.4%	4.9%	4.1%
Panel 2	498	2.9%	11.2%	1.4%	8.2%
Panel 3	2151	4.5%	9.0%	1.2%	5.8%
Panel 4*	5783	5.0%	7.5%	1.2%	0.7%

\*Avoid freeze/thaw cycles with CSF samples

**Spike Recovery (Plasma)**

GFAP (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	600 pg/mL	3000 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	61%	64%	63%	58%
CSF 2	56%	52%	54%	

**Spike Recovery (CSF)**

GFAP (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	60,000 pg/mL	300,000 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	94%	90%	92%	91%
CSF 2	91%	91%	91%	

**Dilution Linearity (Plasma)**

Dilution	Endogenous Plasma	Spiked Plasma
MRD (4x)	100%	100%
2x MRD	117%	110%
4x MRD	*	118%
8x MRD	*	126%

\*Values below LLOQ

**Dilution Linearity (CSF)**

Dilution	Endogenous CSF	Spiked CSF
MRD (4x)	100%	100%
2x MRD	102%	97%
4x MRD	*	94%
8x MRD	*	95%
16x MRD	*	92%
32x MRD	*	88%

\*Values below LLOQ

**Curve Storage**

%Bias	Run 1 Curve	Run 2 Curve	Run 3 Curve	Run 4 Curve
Run 1	0.0%	10.4%	5.0%	11.8%
Run 2	9.4%	0.0%	4.9%	1.2%
Run 3	4.7%	5.2%	0.0%	6.5%
Run 4	10.3%	1.2%	6.0%	0.0%

**Drift**

Sample	Plate 1 End/Start	Plate 1+2 End/Start	Plate 1+2+3 End/Start	Within Run CV
Control 1	8.0%	9.3%	0.6%	5.3%
Control 2	28.6%	17.9%	12.7%	8.7%
Panel 1	1.9%	0.5%	0.6%	4.2%
Panel 2	11.1%	10.5%	9.8%	7.6%

**Normal Sample Reading (Human)**

GFAP	Human Samples			
	EDTA Plasma conc (pg/mL)	CV	CSF conc (pg/mL)	CV
1	50.4	4.9%	12215	4%
2	39.4	2.1%	11327	2%
3	53.0	6.3%	34582	4%
4	76.8	6.6%	61757	6%
5	32.9	6.2%	94189	4%
6	60.3	12.5%	16761	3%
7	63.2	3.9%	4291	3%
8	19.9	12.3%	4176	8%
9	51.9	3.5%	5339	11%
10	50.5	9.5%	4901	9%
11	62.3	5.7%	3995	11%
12	32.3	9.5%	10922	4%
13	56.2	11.3%	5499	6%
14	64.3	4.4%	35445	3%
15	52.5	8.6%	17466	2%
16	25.0	3.8%	8355	7%
17	118	3.9%	3825	8%
18	43.9	4.4%	1144	9%
19	181	2.3%	22724	1%
20	43.2	9.3%	5087	10%
21	---	---	9685	2%
22	---	---	5247	9%
23	---	---	13717	2%
24	---	---	9295	7%
25	---	---	478	37%
<b>Mean</b>	<b>58.8 pg/mL</b>		<b>17426 pg/mL</b>	
<b>Median</b>	<b>52.2 pg/ml</b>		<b>9295 pg/mL</b>	
<b>Range</b>	<b>19.9-181 pg/ml</b>		<b>478 -94189 pg/mL</b>	
<b>Detectability</b>	100%		100%	
<b>Quantifiability</b>	100%		92%	

**IX. NF-L Claims Detail**

**LOD Summary**

Neurology 4-Plex E Advantage Kit Assay Characterization		
Analytical LOQ	0.400 pg/mL	n=12, Derived from primary and reference calibration curves with additional 1:2 and 1:4 dilution of CalC and 1:2 dilution of CalB, 2 lots testing across 2 instruments per lot, 12 runs total
Functional LOQ	EDTA Plasma (4x): 1.6 pg/mL CSF (400x): 160 pg/mL	
LOD	0.090 ± 0.050 (0.016 – 0.152 pg/mL)	n=12, Grand mean of 2.5 SD LOD calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
B/A	2.48 ± 0.278 Range: 2.25-3.25	n=12, Grand mean of B/A calculated off primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal A	0.011 ± 0.002 Range: 0.007-0.013	n=12, Grand mean of Cal A from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total
Cal H	11.6 ± 0.884 Range: 10.5-12.8	n=12, Grand mean of Cal H from primary and reference calibration curves, 2 lots testing across 2 instruments per lot, 12 runs total

**LOD Lot 1**

Reagent Lot	Lot 1							
Instrument	HDX-54			HDX-07			AVE	SD
Primary Cal LOD pg/mL	0.095	0.028	0.031	0.115	0.152	0.111	0.089	0.049
Primary Cal B/A	2.2	2.6	2.3	2.7	2.5	3.2	2.59	0.363
Primary Cal A	0.009	0.009	0.009	0.008	0.009	0.007	0.009	0.001
Primary Cal H	11.2	10.5	11.3	11.0	10.5	10.5	10.8	0.370

**LOD Lot 2**

Reagent Lot	Lot 2							
Instrument	HDX-54			HDX-07			AVE	SD
Reference Cal LOD pg/mL	0.002	0.139	0.129	0.138	0.068	0.076	0.092	0.054
Reference Cal B/A	2.3	2.4	2.3	2.3	2.4	2.5	2.36	0.095
Reference Cal A	0.013	0.013	0.013	0.013	0.012	0.012	0.013	0.001
Reference Cal H	12.2	12.8	12.8	12.2	12.4	12.1	12.4	0.313

**LLOQ Lot 1**

% Recovery						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
1.6 pg/mL	5%	3%	5%	6%	4%	11%
0.801 pg/mL	22%	3%	2%	6%	5%	9%
0.519 pg/mL	9%	13%	7%	16%	17%	7%
0.400 pg/mL	13%	18%	9%	9%	15%	6%
0.260 pg/mL	28%	36%	40%	2%	14%	30%
Pooled CV						
Instrument	HDX-54			HDX-07		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
1.6 pg/mL	100%	102%	97%	98%	104%	98%
0.801 pg/mL	97%	104%	101%	104%	108%	120%
0.519 pg/mL	81%	82%	93%	93%	88%	106%
0.400 pg/mL	94%	87%	123%	94%	103%	104%
0.260 pg/mL	119%	84%	98%	130%	122%	109%

**LLOQ Lot 2**

% Recovery						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
1.6 pg/mL	2%	5%	4%	17%	11%	19%
0.801 pg/mL	15%	16%	8%	11%	7%	2%
0.519 pg/mL	5%	6%	6%	7%	11%	20%
0.400 pg/mL	13%	21%	17%	16%	12%	3%
0.260 pg/mL	10%	2%	24%	45%	18%	18%
Pooled CV						
Instrument	HDX-07			HDX-54		
Experiment	RUN01	RUN02	RUN03	RUN01	RUN02	RUN03
1.6 pg/mL	95%	95%	98%	100%	98%	97%
0.801 pg/mL	110%	110%	100%	108%	106%	108%
0.519 pg/mL	113%	95%	98%	92%	100%	107%
0.400 pg/mL	102%	83%	95%	92%	110%	111%
0.260 pg/mL	120%	104%	91%	62%	115%	140%

**LLOQ Summary**

LOQ Point	LOQ Point (pg/mL)	Pooled CV	Mean Recovery	LLOQ Claim (pg/mL)
High	1.60	9.7%	98.4%	<b>0.400</b>
Mid-High	0.801	11.8%	95.7%	
Mid	0.519	11.1%	106%	
Low-Mid	0.400	14.2%	99.9%	
Low	0.260	27.1%	108%	

**Precision Sample Results**

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	20.1	4.5%	5.2%	1.8%	5.62%
Control 2	410	5.3%	6.4%	3.62%	1.48%
Panel 1	6.29	8.1%	7.2%	1.3%	5.9%
Panel 2	70.0	2.9%	7.2%	1.5%	7.8%
Panel 3	360	4.4%	7.5%	2.3%	5.4%
Panel 4	299	10.8%	10.3%	4.4%	0.2%

**Spike Recovery (Plasma)**

NF-L (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	150 pg/mL	750 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	118%	118%	118%	111%
CSF 2	109%	100%	105%	

**Spike Recovery (CSF)**

NF-L (w/ Buffer Correction) % Recovery [(Spiked Matrix - Unspiked Matrix) / Spiked Buffer Conc] x 100				
Sample	15,000 pg/mL	75,000 pg/mL	Mean	Grand Mean
Diluent	100%	100%	-	-
CSF 1	88%	89%	92%	89%
CSF 2	86%	91%	91%	

**Dilution Linearity (Plasma)**

Dilution	Endogenous Plasma	Spiked Plasma
MRD (4x)	100%	100%
2x MRD	111%	100%
4x MRD	*	95%
8x MRD	*	95%

\*Values below LLOQ

**Dilution Linearity (CSF)**

Dilution	Endogenous CSF	Spiked CSF
MRD (4x)	100%	100%
2x MRD	91%	93%
4x MRD	*	96%
8x MRD	*	93%
16x MRD	*	92%
32x MRD	*	88%

\*Values below LLOQ

**Curve Storage**

%Bias	Run 1 Curve	Run 2 Curve	Run 3 Curve	Run 4 Curve
Run 1	0.0%	1.8%	2.9%	0.2%
Run 2	1.7%	0.0%	4.6%	2.0%
Run 3	2.9%	4.7%	0.0%	2.6%
Run 4	0.3%	2.1%	2.6%	0.0%

**Drift**

Sample	Plate 1 End/Start	Plate 1+2 End/Start	Plate 1+2+3 End/Start	Within Run CV
Control 1	3.8%	1.0%	0.0%	4.5%
Control 2	30.1%	19.1%	15.1%	8.7%
Panel 1	2.3%	6.6%	9.9%	4.5%
Panel 2	18.3%	17.7%	19.6%	9.1%

**Normal Sample Reading (Human)**

NF-L	Human Samples			
	EDTA Plasma conc (pg/mL)	CV	CSF conc (pg/mL)	CV
1	4.09	7.4%	1232	4%
2	3.09	15.4%	5818	2%
3	49.0	2.9%	679	8%
4	29.6	1.4%	2810	4%
5	3.77	10.9%	6064	2%
6	12.4	9.7%	2738	4%
7	17.8	6.3%	1842	1%
8	2.77	7.0%	4272	6%
9	10.81	3.0%	249	5%
10	8.39	5.7%	276	10%
11	5.46	6.9%	1646	5%
12	4.46	6.2%	1268	7%
13	13.9	3.4%	755	4%
14	10.8	3.2%	437	9%
15	7.90	4.2%	25722	8%
16	3.00	9.6%	1491	4%
17	32.5	4.3%	602	3%
18	25.3	5.1%	552	3%
19	17.7	0.9%	1394	2%
20	9.22	5.9%	3702	1%
21	---	---	2029	3%
22	---	---	1241	4%
23	---	---	519	6%
24	---	---	674	11%
25	---	---	561	6%
<b>Mean</b>	<b>13.6 pg/ml</b>		<b>2743 pg/mL</b>	
<b>Median</b>	<b>10.0 pg/ml</b>		<b>1268 pg/mL</b>	
<b>Range</b>	<b>2.77-49.0 pg/ml</b>		<b>249 -25722 pg/mL</b>	
<b>Detectability</b>	100%		100%	
<b>Quantifiability</b>	100%		100%	