

This study report demonstrates the Simoa® NPTX2 Advantage PLUS assay properties on Simoa® HD-X for the generation of claims data. Data provided here includes Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), precision, spike recovery, dilution linearity, curve storage, drift, and normal sample range.

## I. NPTX2 Advantage PLUS Kit Summary

| Test                                 | Claim                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| LOD                                  | 0.8646 ± 0.8312 pg/mL<br>Range: 0.1266 - 2.7531 pg/mL                                                                                                                                                                                                                                                                                 |
| Analytical LLOQ<br>(Fit For Purpose) | 2.9297 pg/mL                                                                                                                                                                                                                                                                                                                          |
| Functional LLOQ                      | CSF (8x): 23.4376 pg/mL<br>Serum/EDTA Plasma (32x): 93.7504 pg/mL                                                                                                                                                                                                                                                                     |
| Calibrator B/A                       | 1.8404 ± 0.4472<br>Range: 0.9130 - 2.4252                                                                                                                                                                                                                                                                                             |
| Calibrator A                         | 0.0044 ± 0.0014<br>Range: 0.0028 - 0.0073                                                                                                                                                                                                                                                                                             |
| Calibrator H                         | 11.5912 ± 0.6557<br>Range: 9.8300 - 14.5631                                                                                                                                                                                                                                                                                           |
| Precision                            | Within Run CV: 3.7%<br>Run to Run Conc CV: 11.4%<br>Instrument CV: 5.8%<br>Lot CV: 5.2%                                                                                                                                                                                                                                               |
| Spike Recovery                       | CSF: 90.4%, Range: 85.1 - 105.3%<br>Serum: 84.9%, Range: 72.6 - 89.2%<br>EDTA Plasma: 72.4%, Range: 64.2 - 77.5%                                                                                                                                                                                                                      |
| Dilution Linearity                   | CSF (Linear Range 4x-64x): 110.2%, Range: 87.5 – 164.0%<br>Serum (Linear Range 16x-64x): 98.8%, Range: 76.5 - 123.9%<br>EDTA Plasma (Linear Range 16x-64x): 97.7%, Range: 75.2 - 116.8%                                                                                                                                               |
| Curve Storage                        | Bias: 5.6%, Range: 4.3 - 8.0%                                                                                                                                                                                                                                                                                                         |
| Drift                                | Three-plate Variance: Plate 1: 4%; Plate 1+2: 9.1%; Plate 1+2+3: 6.3%<br>Three-Plate Precision: 4.6%, Range: 3.6 - 5.5%                                                                                                                                                                                                               |
| Normal Samples                       | Mean CSF: 2624.4494 pg/mL, Range: 689.8556 - 7286.7503,<br>Detectability: 100%, Quantifiability: 100%<br>Mean Serum: 7455.5185 pg/mL, Range: 3518.4901 - 17016.6132,<br>Detectability: 100%, Quantifiability: 100%<br>Mean EDTA Plasma: 5945.6154 pg/mL, Range: 2954.3680 - 12783.5311,<br>Detectability: 100%, Quantifiability: 100% |

## II. 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:<br>0.8646 pg/mL<br><br>Range: 0.1266 - 2.7531 pg/mL |
| # Instruments                     | 2  |                                                                     |
| Runs/Instrument                   | 6  |                                                                     |
| # 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  $\leq 20\%$  pooled CV and 80 – 120% mean recovery over all runs. Functional LLOQ is the Analytical LLOQ multiplied by the recommended dilution.

| Test Conditions                   |                                                                                                | Claim                                                         |
|-----------------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| Samples                           | Lyophilized Calibrator Concentrate diluted to 2.9297, 1.4648, 0.9766, 0.7324, and 0.3662 pg/mL | Analytical LLOQ:<br>2.9297 pg/mL                              |
| Replicates/Run (each test sample) | 3                                                                                              | Functional LLOQ:<br>CSF (8x):<br>23.4376 pg/mL                |
| # Instruments                     | 2                                                                                              |                                                               |
| Runs/Instrument                   | 6                                                                                              |                                                               |
| # Lots Tested                     | 2                                                                                              |                                                               |
| Total Runs                        | 12                                                                                             | Functional LLOQ:<br>Serum/EDTA Plasma (32x):<br>93.7504 pg/mL |

### Precision Profile:

Artificial CSF spiked with antigen and 2 controls were diluted 1:8 in sample diluent; Commercial pooled serum samples and commercial pooled plasma samples were diluted 1:32 in sample diluent. 3 runs were performed on 2 instruments using 2 reagent lots. 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<br>Control 2: High Concentration Calibrator spiked into calibrator diluent<br>CSF 1: Artificial CSF spiked with antigen<br>CSF 2: Artificial CSF spiked with antigen<br>Serum 1: Commercial pooled serum spiked with antigen<br>Serum 2: Commercial pooled serum spiked with antigen<br>Plasma 1: Commercial pooled plasma spiked with antigen<br>Plasma 2: Commercial pooled plasma spiked with antigen | Mean Total CV: 6.5% |
| Specifications     | Replicates/Sample: 3<br>Instruments: 2<br>Runs/Instrument: 6<br>Lots Tested: 2<br>Total Runs: 12<br>Total Measurements/Sample: 36                                                                                                                                                                                                                                                                                                                                               |                     |
| Within Run CV      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 3.7%                |
| Run to Run Conc CV |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 11.4%               |
| Instrument CV      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 5.8%                |
| Lot CV             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 5.2%                |

### Spike Recovery (CSF, Serum, and EDTA Plasma):

6 normal human samples (2 CSF, 2 serum, and 2 EDTA plasma) and a diluent control were spiked at 2 concentrations (200 pg/mL and 1000 pg/mL) with NPTX2 antigen and then diluted 1:8 (CSF) and 1:32 (serum and EDTA plasma) with sample diluent. Samples were tested with two reagent lots. Percent recovery is defined as the difference between the measured concentration of NPTX2 in the spiked sample and the measured concentration in unspiked sample relative to the concentration of NPTX2 in spiked sample diluent.

| Test Conditions                   |                                                                   | Claim                                                              |
|-----------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------|
| Test Samples                      | Normal Human Samples - 2 CSF                                      | Buffer Corrected Grand Mean CSF: 90.4%<br><br>Range: 85.1 - 105.3% |
| Spike Concentrations              | Control: Calibrator Diluent<br>Low: 200 pg/mL<br>High: 1000 pg/mL |                                                                    |
| Replicates/Run (each test sample) | 3                                                                 |                                                                    |

| Test Conditions                      |                                                                   | Claim                                                                     |
|--------------------------------------|-------------------------------------------------------------------|---------------------------------------------------------------------------|
| Test Samples                         | Normal Human Samples - 2 Serum                                    | Buffer Corrected<br>Grand Mean Serum:<br>84.9%<br><br>Range: 72.6 - 89.2% |
| Spike Concentrations                 | Control: Calibrator Diluent<br>Low: 200 pg/mL<br>High: 1000 pg/mL |                                                                           |
| Replicates/Run<br>(each test sample) | 3                                                                 |                                                                           |

| Test Conditions                      |                                                                   | Claim                                                                           |
|--------------------------------------|-------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Test Samples                         | Normal Human Samples - 2 EDTA Plasma                              | Buffer Corrected<br>Grand Mean EDTA Plasma:<br>72.4%<br><br>Range: 64.2 - 77.5% |
| Spike Concentrations                 | Control: Calibrator Diluent<br>Low: 200 pg/mL<br>High: 1000 pg/mL |                                                                                 |
| Replicates/Run<br>(each test sample) | 3                                                                 |                                                                                 |

#### Dilution Linearity (CSF, Serum, and EDTA Plasma):

12 unspiked normal human samples (4 CSF, 4 serum, and 4 EDTA plasma) were diluted 2x serially with sample diluent from 4x to 512x for CSF, and 8x to 1024x for serum and EDTA plasma. The average percent recovery across the linear range is displayed for each sample type.

| Test Conditions                      |                          | Claim                                                                                |
|--------------------------------------|--------------------------|--------------------------------------------------------------------------------------|
| Test Samples                         | 4 Normal Human CSF       | Mean Endogenous<br>CSF Dilution Linearity (4x - 64x): 110.2%<br>Range: 87.5 – 164.0% |
| Dilutions                            | 2x serial from 4x to 64x |                                                                                      |
| Replicates/Run<br>(each test sample) | 3                        |                                                                                      |

| Test Conditions                      |                                                        | Claim                                                                                                                                                                                      |
|--------------------------------------|--------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Samples                         | 4 Normal Human Serum and<br>4 Normal Human EDTA Plasma | Mean Endogenous<br>Serum Dilution Linearity (16x - 64x): 98.8%<br>Range: 76.5 - 123.9%<br><br>Mean Endogenous<br>EDTA Plasma Dilution Linearity (16x - 64x): 97.7%<br>Range: 75.2 - 116.8% |
| Dilutions                            | 2x serial from 16x to 64x                              |                                                                                                                                                                                            |
| Replicates/Run<br>(each test sample) | 3                                                      |                                                                                                                                                                                            |

### Curve Storage:

Artificial CSF spiked with antigen and 2 controls were diluted 1:8 in sample diluent. Commercial pooled serum samples and commercial pooled plasma samples were diluted 1:32 in sample diluent. 6 runs were performed on 5 individual days (5 days total) on the same instrument using 1 lot of reagents. The mean total bias is determined by the concentration bias for each CSF sample, serum sample, plasma sample, and control derived from each of other 5 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<br>Control 2: High Concentration Calibrator spiked into calibrator diluent<br>CSF 1: Artificial CSF spiked with antigen<br>CSF 2: Artificial CSF spiked with antigen<br>Serum 1: Commercial pooled serum spiked with antigen<br>Serum 2: Commercial pooled serum spiked with antigen<br>Plasma 1: Commercial pooled plasma spiked with antigen<br>Plasma 2: Commercial pooled plasma spiked with antigen | Mean Total Bias:<br>5.6%<br><br>Range:<br>4.3 – 8.0% |
| Specifications  | Replicates/Sample: 3<br>Instruments: 1<br>Runs/Instrument: 6<br>Lots Tested: 1<br>Total Runs: 6                                                                                                                                                                                                                                                                                                                                                                                 |                                                      |

### Drift:

2 artificial CSF samples spiked with antigen and 2 controls were run across three plates with a total of 54 replicates per sample. The samples were diluted 1:8 in sample diluent. 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<br>Control 2: High Concentration Calibrator spiked into calibrator diluent<br>CSF 1: Artificial CSF spiked with antigen<br>CSF 2: Artificial CSF spiked with antigen | Three-plate Variance:<br>Plate 1: 4%<br>Plate 1+2: 9.1%<br>Plate 1+2+3: 6.3% |
| Specifications  | Replicates/Sample: 3<br>Replicates of Sample/Plate: 6<br>Plates: 3<br>Total Replicates: 54                                                                                                                                                  | Precision:<br><br>5.5%                                                       |

**Normal Sample Reading: (CSF, Serum, and EDTA Plasma)**

19 normal human CSF samples were diluted 1:8 with sample diluent and 20 matched normal human serum and EDTA plasma samples were diluted 1:32 with sample diluent and were evaluated using 2 lots of reagents. The mean concentrations in pg/mL for each sample type are displayed in the table below.

| Test Conditions                   |                                                                       | Claim                                                                                                                                                                                                                                    |
|-----------------------------------|-----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Samples                      | 19 Normal Human CSF and 20 Matched Normal Human Serum and EDTA Plasma | Mean CSF: 2624.4494 pg/mL<br>Range: 689.8556 - 7286.7503<br>Detectability: 100%<br>Quantifiability: 100 %                                                                                                                                |
| Replicates/Lot (each test sample) | 3                                                                     | Mean Serum: 7455.5185 pg/mL<br>Range: 3518.4901 - 17016.6132<br>Detectability: 100%<br>Quantifiability: 100 %<br><br>Mean EDTA Plasma: 5945.6154 pg/mL<br>Range: 2954.3680 - 12783.5311<br>Detectability: 100%<br>Quantifiability: 100 % |

### III. Claims Detail

#### LOD and LOQ Summary

| NPTX2 Advantage PLUS Kit Assay Characterization |                                                                   |                                                                                                                                                                              |
|-------------------------------------------------|-------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>LOD</b>                                      | 0.8646 ± 0.8312 pg/mL<br>(0.1266 - 2.7531 pg/mL)                  | n=12, Mean of 2.5 SD LOD calculated from calibration curves, 2 lots tested across 3 instruments per lot, 12 runs total                                                       |
| <b>Analytical LLOQ</b>                          | 2.9297 pg/mL                                                      | n=12, Derived from Lyophilized Calibrator Concentrate diluted to 2.9297, 0.7324, 1.4648, 0.9766, and 0.3662 pg/mL. 2 lots tested across 3 instruments per lot, 12 runs total |
| <b>Functional LLOQ</b>                          | CSF (8x): 23.4376 pg/mL<br>Serum/EDTA Plasma (32x): 93.7504 pg/mL |                                                                                                                                                                              |
| <b>B/A</b>                                      | 1.8404 ± 0.4472<br>(0.9130 - 2.4252)                              | n=12, Mean of B/A calculated from calibration curves, 2 lots tested across 3 instruments per lot, 12 runs total                                                              |
| <b>Cal A</b>                                    | 0.0044 ± 0.0014<br>(0.0028 - 0.0073)                              | n=12, Mean of Cal A from calibration curves, 2 lots tested across 3 instruments per lot, 12 runs total                                                                       |
| <b>Cal H</b>                                    | 11.5912 ± 0.6557<br>(9.8300 - 14.5631)                            | n=12, Mean of Cal H from calibration curves, 2 lots tested across 3 instruments per lot, 12 runs total                                                                       |

#### LOD Lot 1

| Reagent Lot      | Lot 1   |         |         |         |         |         |         |        |
|------------------|---------|---------|---------|---------|---------|---------|---------|--------|
| Run              | 1       | 2       | 3       | 4       | 5       | 6       | AVE     | SD     |
| <b>LOD pg/mL</b> | 0.5865  | 0.5351  | 0.5006  | 2.0823  | 1.2293  | 0.1266  | 0.8434  | 0.7037 |
| <b>Cal B/A</b>   | 2.3179  | 1.7756  | 1.6899  | 1.4252  | 2.4252  | 1.7890  | 1.9038  | 0.3867 |
| <b>Cal A</b>     | 0.0037  | 0.0045  | 0.0046  | 0.0073  | 0.0041  | 0.0041  | 0.0047  | 0.0013 |
| <b>Cal H</b>     | 14.5631 | 12.1700 | 12.5520 | 12.3860 | 12.0377 | 13.2201 | 12.8215 | 0.9479 |

#### LOD Lot 2

| Reagent Lot      | Lot 2   |         |         |         |         |        |         |        |
|------------------|---------|---------|---------|---------|---------|--------|---------|--------|
| Run              | 1       | 2       | 3       | 4       | 5       | 6      | AVE     | SD     |
| <b>LOD pg/mL</b> | 0.1782  | 0.7866  | 2.7531  | 0.4481  | 0.2490  | 0.8997 | 0.8858  | 0.9586 |
| <b>Cal B/A</b>   | 2.0926  | 1.4332  | 0.9130  | 1.9304  | 2.0300  | 2.2634 | 1.7771  | 0.5076 |
| <b>Cal A</b>     | 0.0028  | 0.0042  | 0.0072  | 0.0036  | 0.0035  | 0.0039 | 0.0042  | 0.0015 |
| <b>Cal H</b>     | 10.8336 | 10.1408 | 10.6794 | 10.3882 | 10.2939 | 9.8300 | 10.3610 | 0.3634 |

### Analytical LLOQ Lot 1

| % Recovery |        |        |        |        |        |        |
|------------|--------|--------|--------|--------|--------|--------|
| Run        | 1      | 2      | 3      | 4      | 5      | 6      |
| 2.9297     | 97.1%  | 96.4%  | 96.2%  | 102.3% | 88.5%  | 109.3% |
| 1.4648     | 95.4%  | 116.3% | 109.9% | 75.3%  | 111.0% | 133.3% |
| 0.9766     | 110.7% | 104.0% | 79.3%  | 69.7%  | 97.4%  | 126.4% |
| 0.7324     | 111.9% | 96.3%  | 105.9% | 80.3%  | 137.4% | 85.7%  |
| 0.3662     | 132.8% | 287.9% | 186.9% | 154.6% | 68.2%  | 240.1% |
| Pooled CV  |        |        |        |        |        |        |
| Run        | 1      | 2      | 3      | 4      | 5      | 6      |
| 2.9297     | 12.8%  | 3.8%   | 14.1%  | 29.6%  | 6.7%   | 10.3%  |
| 1.4648     | 36.5%  | 15.8%  | 44.2%  | 21.0%  | 2.0%   | 23.9%  |
| 0.9766     | 38.4%  | 39.4%  | 45.0%  | 44.9%  | 13.0%  | 22.7%  |
| 0.7324     | 23.3%  | 27.4%  | 64.6%  | 69.7%  | 75.5%  | 3.5%   |
| 0.3662     | 78.2%  | 133.2% | 85.9%  | 80.4%  | 92.1%  | 67.2%  |

### Analytical LLOQ Lot 2

| % Recovery |        |        |        |        |        |        |
|------------|--------|--------|--------|--------|--------|--------|
| Run        | 1      | 2      | 3      | 4      | 5      | 6      |
| 2.9297     | 103.8% | 104.3% | 99.8%  | 123.6% | 117.2% | 85.2%  |
| 1.4648     | 106.4% | 144.4% | 82.9%  | 96.4%  | 131.1% | 78.8%  |
| 0.9766     | 137.6% | 111.5% | 128.3% | 110.6% | 108.8% | 107.0% |
| 0.7324     | 91.2%  | 74.6%  | 59.6%  | 80.4%  | 89.4%  | 150.5% |
| 0.3662     | 163.3% | 94.6%  | 132.0% | 220.8% | 222.3% | 73.8%  |
| Pooled CV  |        |        |        |        |        |        |
| Run        | 1      | 2      | 3      | 4      | 5      | 6      |
| 2.9297     | 24.3%  | 13.5%  | 31.1%  | 4.7%   | 17.0%  | 6.7%   |
| 1.4648     | 16.9%  | 37.4%  | 72.5%  | 22.8%  | 36.1%  | 15.7%  |
| 0.9766     | 38.7%  | 13.5%  | 71.8%  | 31.1%  | 15.3%  | 58.7%  |
| 0.7324     | 25.9%  | 70.6%  | 96.1%  | 16.1%  | 48.9%  | 49.7%  |
| 0.3662     | 25.7%  | 48.5%  | 52.3%  | 98.4%  | 78.9%  | 65.5%  |

### Analytical LLOQ Summary

| LOQ Target    | Pooled CV    | Mean Recovery | LLOQ Claim (pg/mL) |
|---------------|--------------|---------------|--------------------|
| <b>2.9297</b> | <b>17.8%</b> | <b>102.0%</b> | 2.9297             |
| 1.4648        | 35.1%        | 106.8%        |                    |
| 0.9766        | 41.8%        | 107.6%        |                    |
| 0.7324        | 57.2%        | 96.9%         |                    |
| 0.3662        | 83.5%        | 164.8%        |                    |

### Precision Sample Results

| Sample    | Mean (pg/mL) | Within Run CV | Run to Run Run Conc CV | Instrument CV | Lot CV |
|-----------|--------------|---------------|------------------------|---------------|--------|
| Control 1 | 215.4706     | 4.3%          | 12.9%                  | 3.5%          | 2.5%   |
| Control 2 | 5346.4157    | 3.7%          | 10.0%                  | 8.4%          | 0.3%   |
| CSF 1     | 333.0763     | 3.1%          | 11.3%                  | 4.8%          | 2.5%   |
| CSF 2     | 14640.7902   | 2.6%          | 11.1%                  | 8.5%          | 3.6%   |
| Serum 1   | 5546.7314    | 3.5%          | 7.4%                   | 6.4%          | 0.7%   |
| Serum 2   | 28475.9765   | 3.4%          | 8.6%                   | 4.8%          | 5.6%   |
| Plasma 1  | 224.1186     | 6.1%          | 13.7%                  | 7.5%          | 10.6%  |
| Plasma 2  | 5079.3417    | 2.7%          | 16.5%                  | 2.3%          | 15.6%  |

### Spike Recovery (CSF)

| NPTX2 (w/ Buffer Correction) % Recovery<br>[(Spiked CSF - Unspiked CSF)/Spiked Buffer Conc] x 100 |           |           |            |             |
|---------------------------------------------------------------------------------------------------|-----------|-----------|------------|-------------|
| Reagent Lot                                                                                       | Sample ID | 200 pg/mL | 1000 pg/mL | Grand Mean* |
| Lot 1                                                                                             | Diluent*  | 100.0%    | 100.0%     | 90.4%       |
|                                                                                                   | CSF 1     | 93.3%     | 105.3%     |             |
|                                                                                                   | CSF 2     | 88.5%     | 82.0%      |             |
| Lot 2                                                                                             | Diluent*  | 99.9%     | 100.0%     |             |
|                                                                                                   | CSF 1     | 84.3%     | 96.0%      |             |
|                                                                                                   | CSF 2     | 85.1%     | 89.0%      |             |

\*Diluent results excluded from Grand Mean calculation

### Spike Recovery (Serum)

| NPTX2 (w/ Buffer Correction) % Recovery<br>[(Spiked Serum - Unspiked Serum)/Spiked Buffer Conc] x 100 |           |           |            |             |
|-------------------------------------------------------------------------------------------------------|-----------|-----------|------------|-------------|
| Reagent Lot                                                                                           | Sample ID | 200 pg/mL | 1000 pg/mL | Grand Mean* |
| Lot 1                                                                                                 | Diluent*  | 99.8%     | 100.0%     | 84.9%       |
|                                                                                                       | Serum 1   | 85.2%     | 88.1%      |             |
|                                                                                                       | Serum 2   | 85.1%     | 88.7%      |             |
| Lot 2                                                                                                 | Diluent*  | 99.8%     | 100.0%     |             |
|                                                                                                       | Serum 1   | 89.2%     | 83.6%      |             |
|                                                                                                       | Serum 2   | 86.8%     | 72.6%      |             |

\*Diluent results excluded from Grand Mean calculation

### Spike Recovery (EDTA Plasma)

| NPTX2 (w/ Buffer Correction) % Recovery<br>[(Spiked EDTA Plasma - Unspiked EDTA Plasma)/Spiked Buffer Conc] x 100 |               |           |            |             |
|-------------------------------------------------------------------------------------------------------------------|---------------|-----------|------------|-------------|
| Reagent Lot                                                                                                       | Sample ID     | 200 pg/mL | 1000 pg/mL | Grand Mean* |
| Lot 1                                                                                                             | Diluent*      | 99.8%     | 100.0%     | 72.4%       |
|                                                                                                                   | EDTA Plasma 1 | 64.2%     | 70.8%      |             |
|                                                                                                                   | EDTA Plasma 2 | 74.7%     | 75.5%      |             |
| Lot 2                                                                                                             | Diluent*      | 99.8%     | 100.0%     |             |
|                                                                                                                   | EDTA Plasma 1 | 70.2%     | 74.6%      |             |
|                                                                                                                   | EDTA Plasma 2 | 77.5%     | 72.0%      |             |

\*Diluent results excluded from Grand Mean calculation

### Dilution Linearity (CSF)

|          | Lot 1  |        |        |        | Lot 2  |        |        |        |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| Dilution | CSF 1  | CSF 2  | CSF 3  | CSF 4  | CSF 1  | CSF 2  | CSF 3  | CSF 4  |
| 4x       | 97.7%  | 96.9%  | 89.4%  | 115.4% | 92.5%  | 94.0%  | 87.7%  | 87.5%  |
| 8x       | 100%   | 100%   | 100%   | 100%   | 100%   | 100%   | 100%   | 100%   |
| 16x      | 110.0% | 111.8% | 105.5% | 136.4% | 106.0% | 109.1% | 108.0% | 107.4% |
| 32x      | 107.3% | 112.9% | 109.7% | 136.4% | 103.6% | 112.2% | 104.8% | 109.5% |
| 64x      | 114.5% | 112.0% | 116.3% | 164.0% | 111.3% | 118.3% | 116.9% | 121.0% |
| 128x     | 120.2% | 124.7% | 114.0% | 164.7% | 114.9% | 123.0% | 110.8% | 123.9% |
| 256x     | < LOQ  | 124.8% | 106.4% | 144.1% | < LOQ  | 114.4% | 101.1% | 126.0% |
| 512x     | < LOQ  | < LOQ  | < LOQ  | < LOQ  | < LOQ  | < LOQ  | <LOQ   | < LOQ  |

### Dilution Linearity (Serum)

|          | Lot 1   |         |         |         | Lot 2   |         |         |         |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|
| Dilution | Serum 1 | Serum 2 | Serum 3 | Serum 4 | Serum 1 | Serum 2 | Serum 3 | Serum 4 |
| 8x       | 74.8%   | 74.9%   | 89.4%   | 83.2%   | 75.9%   | 71.9%   | 77.0%   | 70.3%   |
| 16x      | 82.8%   | 87.0%   | 99.0%   | 79.9%   | 85.8%   | 76.5%   | 91.6%   | 79.5%   |
| 32x      | 100%    | 100%    | 100%    | 100%    | 100%    | 100%    | 100%    | 100%    |
| 64x      | 112.1%  | 103.3%  | 123.9%  | 107.0%  | 107.1%  | 114.7%  | 114.4%  | 116.4%  |
| 128x     | 114.4%  | 115.2%  | 126.4%  | 120.5%  | 118.0%  | 123.7%  | 130.0%  | 131.1%  |
| 256x     | 119.4%  | 122.2%  | 127.7%  | 135.8%  | 125.9%  | 125.6%  | 141.8%  | 142.1%  |
| 512x     | 127.4%  | < LOQ   | 137.9%  | 129.8%  | 136.2%  | < LOQ   | 150.9%  | 153.3%  |
| 1024x    | < LOQ   | < LOQ   | < LOQ   | < LOQ   | < LOQ   | < LOQ   | < LOQ   | < LOQ   |

**Dilution Linearity (EDTA Plasma)**

|          | Lot 1         |               |               |               | Lot 2         |               |               |               |
|----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Dilution | EDTA Plasma 1 | EDTA Plasma 2 | EDTA Plasma 3 | EDTA Plasma 4 | EDTA Plasma 1 | EDTA Plasma 2 | EDTA Plasma 3 | EDTA Plasma 4 |
| 8x       | 67.5%         | 70.8%         | 78.3%         | 75.4%         | 82.0%         | 64.3%         | 74.7%         | 74.0%         |
| 16x      | 89.9%         | 79.3%         | 88.1%         | 75.2%         | 90.6%         | 79.6%         | 83.5%         | 84.7%         |
| 32x      | 100%          | 100%          | 100%          | 100%          | 100%          | 100%          | 100%          | 100%          |
| 64x      | 106.5%        | 108.3%        | 110.3%        | 107.7%        | 115.6%        | 115.5%        | 111.5%        | 116.8%        |
| 128x     | 133.2%        | 116.6%        | 124.9%        | 138.4%        | 136.0%        | 126.3%        | 129.3%        | 141.8%        |
| 256x     | 144.3%        | 133.4%        | 138.2%        | 136.5%        | 152.3%        | 143.8%        | 137.3%        | 144.1%        |
| 512x     | 153.6%        | 121.8%        | 137.5%        | 144.2%        | 168.4%        | 160.6%        | 146.2%        | 160.0%        |
| 1024x    | < LOQ         | < LOQ         | < LOQ         | < LOQ         | < LOQ         | < LOQ         | < LOQ         | < LOQ         |

Curve Storage

| %Bias | Run 1 Curve | Run 2 Curve | Run 3 Curve | Run 4 Curve | Run 5 Curve | Run 6 Curve |
|-------|-------------|-------------|-------------|-------------|-------------|-------------|
| Run 1 | 0%          | 4.5%        | 3.9%        | 2.7%        | 3.7%        | 8.0%        |
| Run 2 | 4.7%        | 0%          | 8.6%        | 2.0%        | 8.6%        | 13.0%       |
| Run 3 | 3.4%        | 7.8%        | 0%          | 5.9%        | 0.1%        | 4.2%        |
| Run 4 | 2.8%        | 2.0%        | 6.9%        | 0%          | 6.7%        | 10.8%       |
| Run 5 | 3.4%        | 7.8%        | 0.1%        | 5.9%        | 0%          | 4.2%        |
| Run 6 | 7.3%        | 11.6%       | 3.6%        | 9.7%        | 3.8%        | 0%          |

Drift

| Sample    | Plate 1 End/Start | Plate 1+2 End/Start | Plate 1+2+3 End/Start | Within Run CV |
|-----------|-------------------|---------------------|-----------------------|---------------|
| Control 1 | 5.6%              | 4.7%                | 5.3%                  | 5.5%          |
| Control 2 | 3.2%              | 13.8%               | 11.3%                 | 4.6%          |
| CSF 1     | 0.6%              | 2.5%                | 0.3%                  | 3.6%          |
| CSF 2     | 6.4%              | 15.4%               | 8.3%                  | 4.8%          |

**Normal Sample Reading (Human CSF, Serum, EDTA Plasma)**

|                        | Human Samples (n=6)  |        |                        |       |                             |       |
|------------------------|----------------------|--------|------------------------|-------|-----------------------------|-------|
|                        | CSF conc<br>(pg/mL)  | CV     | Serum conc<br>(pg/mL)  | CV    | EDTA Plasma<br>conc (pg/mL) | CV    |
| 1                      | 7286.7503            | 4.1%   | 11354.2432             | 8.4%  | 6784.2164                   | 3.0%  |
| 2                      | 2176.4446            | 5.5%   | 6620.3448              | 4.1%  | 5551.8576                   | 6.7%  |
| 3                      | 6447.8829            | 3.8%   | 4893.6523              | 3.9%  | 3819.2504                   | 2.3%  |
| 4                      | 2744.9502            | 2.9%   | 17016.6132             | 18.3% | 12783.5311                  | 15.0% |
| 5                      | 2355.6869            | 2.0%   | 7547.3119              | 17.8% | 6263.9481                   | 3.6%  |
| 6                      | 882.1506             | 2.3%   | 11234.7156             | 7.0%  | 9362.9051                   | 3.7%  |
| 7                      | N/A*                 | N/A*   | 7381.1887              | 17.1% | 7199.8397                   | 1.1%  |
| 8                      | 2183.2132            | 6.2%   | 6132.4691              | 15.3% | 4877.1725                   | 6.5%  |
| 9                      | 2267.1904 †          | 4.9% † | 5932.8180              | 17.6% | 4228.1375                   | 4.8%  |
| 10                     | 4708.4698            | 2.0%   | 4690.3712              | 5.6%  | 3963.9290                   | 12.4% |
| 11                     | 2499.1912            | 10.2%  | 12536.9438             | 21.2% | 9698.6061                   | 3.3%  |
| 12                     | 689.8556             | 1.7%   | 6130.0448              | 4.2%  | 4718.7675                   | 5.9%  |
| 13                     | 2185.5329            | 3.2%   | 7151.7355              | 1.7%  | 5167.1283                   | 4.6%  |
| 14                     | 977.4227             | 2.3%   | 4465.8259              | 3.7%  | 3132.5468                   | 3.7%  |
| 15                     | 1502.3721            | 4.6%   | 3518.4901              | 6.9%  | 3225.4905                   | 3.2%  |
| 16                     | 1139.0080            | 1.7%   | 7052.1046              | 3.9%  | 6083.7114                   | 8.2%  |
| 17                     | 2291.2297            | 6.2%   | 4553.8643              | 2.9%  | 2954.3680                   | 4.8%  |
| 18                     | 1963.3552            | 2.6%   | 4388.5735              | 2.9%  | 3883.3595                   | 2.4%  |
| 19                     | 1822.8538            | 3.6%   | 8311.5337              | 10.8% | 7768.7855                   | 3.3%  |
| 20                     | 3740.9794 †          | 2.9%†  | 8197.5248              | 10.0% | 7444.7581                   | 2.8%  |
| <b>Mean</b>            | 2624.4494 pg/mL      |        | 7455.5185 pg/mL        |       | 5945.6154 pg/mL             |       |
| <b>Median</b>          | 2185.5329 pg/mL      |        | 6620.3448 pg/mL        |       | 5359.4930 pg/mL             |       |
| <b>Range</b>           | 689.8556 - 7286.7503 |        | 3518.4901 - 17016.6132 |       | 2954.3680 - 12783.5311      |       |
| <b>Detectability</b>   | 100%                 |        | 100%                   |       | 100%                        |       |
| <b>Quantifiability</b> | 100%                 |        | 100%                   |       | 100%                        |       |

\* Sample was above fULOQ.

† Sample was tested with only one lot of reagents (n=3)