

**Description**

The TAR DNA binding protein of 43 kDa (TDP-43 or TARDBP) is a highly conserved and ubiquitously expressed nuclear protein with roles in transcription and splicing regulation. It is also the major component of ubiquitin-positive cytoplasmic inclusions found in the brains of patients with frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). In addition, TDP-43-containing aggregates are found in a significant number of patients with Alzheimer’s Disease (AD) and other neuromuscular disorders. The majority of TDP-43 protein found in cytoplasmic inclusions is truncated, and it has been shown that the C-terminal domain is intrinsically prone to aggregation. Mutations in the C-terminal region of the TDP-43 gene have been associated with both ALS and FTLD, and are thought to facilitate ubiquitination and phosphorylation of the TDP-43 protein, leading to the formation of pathological inclusions and eventual neurodegeneration. Analysis of TDP-43 levels in plasma may allow the indexing of TDP-43 pathology within the brain to aid in the diagnosis of different forms of dementia and distinguish between TDP-43 proteinopathy and tauopathy. The Simoa TDP-43 assay has been developed with a full-length protein calibrator and antibodies against AA 203 – 209 and the C-terminal region; it is expected to detect both full-length and pathological, truncated forms of the protein.

**Calibration Curve:** Calibrator concentrations and Lower Limit of Quantification depicted.

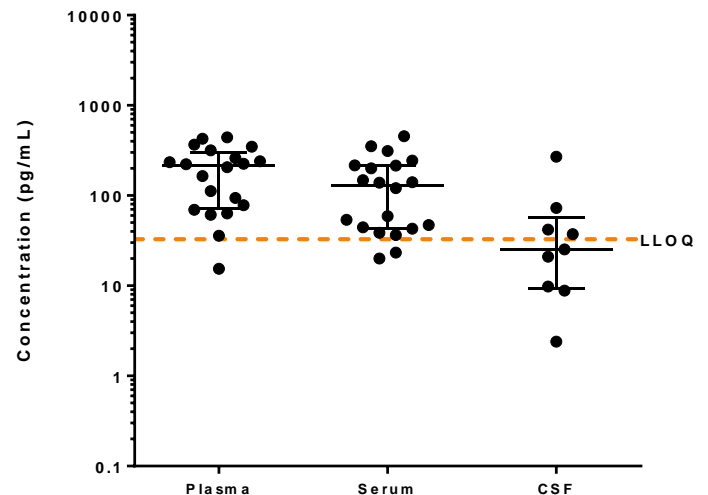
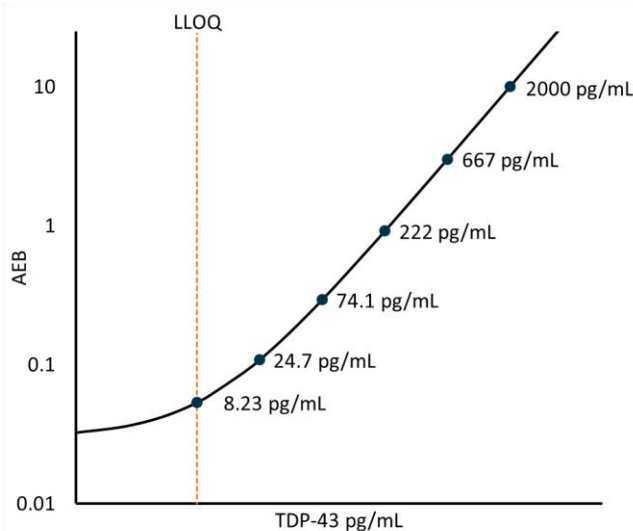
**Lower Limit of Quantification (LLOQ):** Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs each for 2 reagent lot across 2 instruments (12 runs total).

**Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 12 runs each for 2 reagent lot across 2 instruments (12 runs total).

<b>Analytical LLOQ</b>	<b>8.23 pg/mL</b> pooled CV 13% mean recovery 113%
<b>LOD</b>	<b>2.48 pg/mL</b> range 0.234-5.08 pg/mL
<b>Dynamic range (serum and plasma)</b>	0–8000 pg/mL
<b>Diluted Sample volume*</b>	100 µL per measurement
<b>Tests per kit</b>	96

\*See Kit Instruction for details

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=20), and serum (n=20) were measured. Nine CSF samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



Sample Type	Mean TDP-43 pg/mL	Median TDP-43 pg/mL	% Above LOD
Serum	159	130	100%
Plasma	209	214	100%
CSF	127	25.3	69%

**Precision:** Measurements of 1 plasma-based panel, 3 serum-based panels and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 2 reagent lots across 2 instruments (12 runs total, 36 measurements).

Sample	Mean (pg/mL)	Within run CV	Btwn run CV	Btwn inst CV	Btwn lot CV
Control 1	195	4.3%	4.0%	3.4%	1.8%
Control 2	1953	3.1%	4.5%	2.5%	2.5%
Panel 1	59.0	11.8%	11.3%	0.1%	1.1%
Panel 2	73.5	6.2%	14.1%	3.3%	1.3%
Panel 3	316	3.2%	11.1%	7.3%	3.4%
Panel 4	1771	4.0%	8.2%	5.7%	6.0%

**Spike and Recovery:** 2 serum, 2 EDTA plasma and 2 CSF samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

Observed recovery was consistently low in serum and plasma, but results from dilutional linearity and immuno-depletion experiments support specificity of the assay signal.

**Dilution Linearity:** 2 spiked EDTA plasma and 2 spiked serum samples were diluted 2x serially with Sample Diluent from MRD (4x) to 32x. 2 spiked CSF samples were diluted 2x serially with Sample Diluent from MRD (4x) to 64x. All dilutions were performed offline with Sample Diluent and run neat on the HD-1 analyzer. Offline dilution is recommended when assessing dilution linearity for this assay on HD-1/HD-X.

<b>Spike and Recovery (Serum/Plasma)</b>	<b>Mean = 32%</b> Range: 26-42%
<b>Dilution Linearity (Serum/Plasma) (32x)</b>	<b>Mean = 116%</b> Range: 101-135%
<b>Spike and Recovery (CSF)</b>	<b>Mean = 92%</b> Range: 87-100%
<b>Dilution Linearity (CSF) (64x)</b>	<b>Mean = 103%</b> Range: 98-109%

**Immuno-depletion:** 1 Serum and 2 plasma samples were separately incubated with TDP-43 beads and Antibody isotype control beads prior to analysis on HD-1. Mean depletion was 98%.

The Simoa TDP-43 Advantage assay kit is formulated for use on the SR-X®, HD-1, or HD-X® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.