Simoa® Alpha-Synuclein (total) Advantage PLUS Kit HD-X Data Sheet

Item 105767

Description: This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the Simoa® Alpha-Synuclein (total) Advantage PLUS kit on the Simoa HD-X Analyzer®.

Matrix Types: Assay performance and a recommended dilution were evaluated in multiple matrices (Table 1). See the Kit Instructions for sample and dead volume requirements.

Table 1. Minimum recommended dilutions

Matrix Types	Serum , EDTA Plasma, CSF
Diluted Sample Volume	100 uL per measurement
Serum Recommended Dilution	32x
EDTA Plasma Recommended Dilution	32x
CSF Recommended Dilution	8x

Calibration Curve: The reconstitution volume, assigned concentrations, Limit of Detection (LOD), analytical Upper Limit of Quantification (ULOQ), analytical Lower Limit of Quantification (LLOQ) described here are representative and may vary from kit lot to kit lot (Figure 1). Refer to the Certificate of Analysis (CoA) for lot-specific calibrator concentrations and reconstitution volumes.

Limit of Quantification (LOQ): The analytical LLOQ was determined as the lowest concentration of the analyte in Sample Diluent with a recovery between 80 – 120% and a CV < ±20%. The analytical ULOQ (ULOQ) is the concentration of the highest calibrator. The analytical LLOQ and the analytical ULOQ multiplied by the recommended dilution yields the functional LLOQ (fLLOQ) and the functional ULOQ (fLLOQ). The LLOQ was experimentally verified for each kit lot. Minor variations in ULOQ between kit lots may be observed where lot matching was performed (Table 2).

Limit of Detection (LOD): The LOD was calculated as 2.5 standard deviations above the mean of the background (Cal A) (Table 2).

Table 2. LLOQ and LOD

	Analytical	Functional
LLOQ	8.7040 pg/mL	Serum: 278.5280 pg/mL EDTA Plasma: 278.5280 pg/mL CSF: 69.6320 pg/mL
ULOQ	1700.0000 pg/mL	Serum: 54400.0000 pg/mL EDTA Plasma: 54400.0000 pg/mL CSF: 13600.0000 pg/mL
LOD	2.4736 pg/mL	N/A

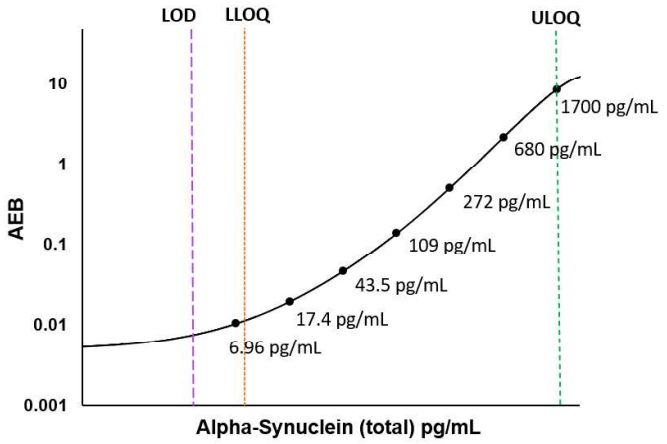

Figure 1. Example calibrator curve

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Reference Ranges: Range was established using normal matched serum (n=20), EDTA plasma (n=20), and CSF (n=20). The mean and median analyte concentrations and the percent of samples above fLLOQ are reported below (Figure 2 and Table 3).

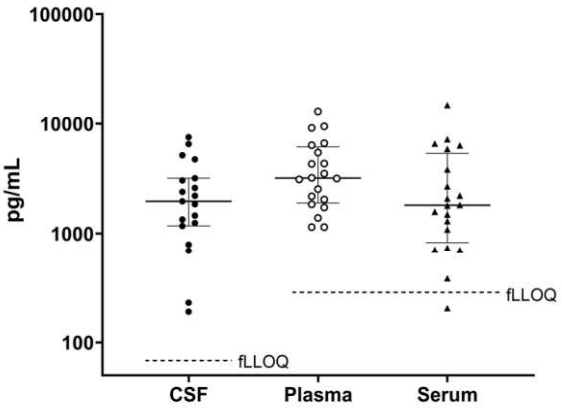

Figure 2. Normal sample readings. Bars depict the median with the interquartile range.

Table 3. Normal sample readings

Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above fLLoQ
Serum	3334.8352	1812.3756	100.0%	95.0%
EDTA Plasma	4289.6046	3193.2052	100.0%	100.0%
CSF *	2543.3548	1969.4589	95.0%	95.0%

* Samples below fLLoQ are excluded from the median and mean

Precision: Precision was calculated using serum, EDTA plasma, CSF, and assay kit controls diluted to the recommended dilution (Table 4). Triplicate measurements were made across 12 runs. Two reagent lots were each tested 3 times on 2 instruments, providing a mean of 36 individual measurements. Four distinct precision values were calculated from the 36 measurements:

1. **Within Run %CV** describes the variability of the %CV within a run, inclusive of 2 reagent lots and 2 instruments.
2. **Run to Run Conc %CV** describes the variability of the concentration value across all 12 runs, inclusive of 2 reagent lots and 2 instruments.
3. **Instrument %CV** describes instrument-to-instrument variability.
4. **Lot %CV** describes lot-to-lot variability.

Table 4. Precision

Sample	Mean (pg/mL)	Within Run %CV	Run to Run Conc %CV	Instrument %CV	Lot %CV
Control 1	2478.3315	9.0%	9.8%	5.5%	5.6%
Control 2	24023.2973	2.3%	4.7%	2.5%	1.2%
Serum 1	818.4599	7.2%	9.5%	5.6%	0.5%
Serum 2	1973.6201	3.8%	7.3%	4.2%	0.7%
Serum 3	22947.2352	6.7%	9.3%	6.5%	2.8%
EDTA Plasma 1	538.7473	10.4%	8.7%	1.4%	2.5%
EDTA Plasma 2	1341.1042	6.4%	6.5%	2.4%	2.9%
EDTA Plasma 3	24160.4529	1.6%	6.9%	3.1%	3.7%

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Spike Recovery: Percent recovery was calculated as the difference between the spiked (with antigen) sample and the un-spiked sample, relative to the spiked (with antigen) Sample Diluent (Table 5).

Linearity: Human serum, EDTA plasma, and CSF samples were diluted 2x serially with Sample Diluent. Linearity refers to the assay's ability to produce proportional and accurate results across a defined dilution range. Linearity was assessed by performing serial dilutions of test samples with Sample Diluent (Table 5).

Table 5. Spike Recovery and Linearity

Spike Recovery (Serum)	**Mean 94.0 %** range 85.5 – 98.9%
Spike Recovery (EDTA Plasma)	**Mean 87.4 %** range 75.4 – 97.5%
Spike Recovery (CSF)	**Mean 112.9 %** range 98.9 – 129.2%
Linearity **(Serum; 8x – 256x)**	**Mean 86.6%** range 34.6-109.3%
Linearity **(EDTA Plasma; 8x – 256x)**	**Mean 94.4 %** range 50.7-135.9%
Linearity **(CSF; 2x – 64x)**	**Mean 81.2 %** range 32.3-106.0%

Other Performance Characteristics: In biologically derived samples (blood/CSF), alpha synuclein is known to be present in multiple conformations, including monomers, oligomers, aggregates and fibrils. The calibrator antigen used in alpha synuclein (total) assay is carefully chosen to represent all these conformations, however, the predominant component is the monomeric alpha synuclein. The Alpha Synuclein (Total) assay is optimized to recognize this variety of conformations in a dose dependent manner. It is critical to note that the antibody reactivity to each type of conformation depends on antigen molar concentration, which is often challenging to determine due to the kinetic nature of the aggregation.

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