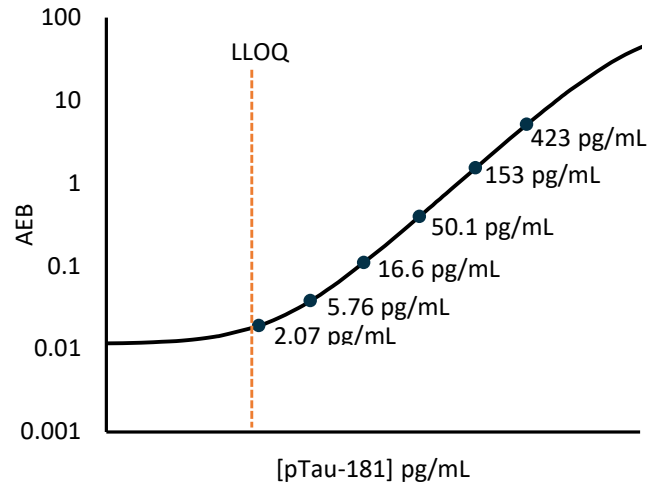


Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the pTau-181 Advantage V2.1 kit on the HD-1/HD-X platform.

Threonine 181 is one of the phosphorylation sites of human tau protein (pTau-181). Tau is a microtubule-stabilizing protein primarily localized in neurons of the central nervous system but also expressed at low levels in astrocytes and oligodendrocytes. Tau consists of six isoforms in the human brain with molecular weights of 48,000 to 67,000 Daltons, depending on isoform. The Simoa pTau-181 assay targets the proline rich region of the Tau protein which is highly conserved amongst these isoforms. Tau elevation is observed in the cerebrospinal fluid (CSF) of patients with neurodegenerative disease and severe head injuries, suggesting its extracellular release during neuronal damage and a role as a biomarker with specificity for brain injury. In Alzheimer’s disease (AD) and related neurodegenerative diseases, including chronic traumatic encephalopathy, tau is abnormally phosphorylated and aggregated into bundles of filaments. pTau-181 has been found to be more strongly associated with markers of AD than total tau.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. Concentrations of Reference Calibrators provided with individual kit lots will vary, as they are value assigned to maintain consistent calibration and sample readings across lots. For pTau-181 V2.1 Reference Calibrator sets, the minimum allowable concentration for Cal G is 320 pg/mL and the maximum allowable concentration for Cal B is 3.09 pg/mL.



Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Human Serum, Plasma & Control Dilution	1:4
Human CSF Dilution	1:10
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs across 2 reagent lots and 2 instruments (3 runs per lot, per instrument). The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV < 20%. The functional LLOQ values represent the analytical LLOQ multiplied by the dilution factor used for the samples. The functional LLOQ for plasma/serum and CSF are 4x and 10x the analytical LLOQ, respectively.

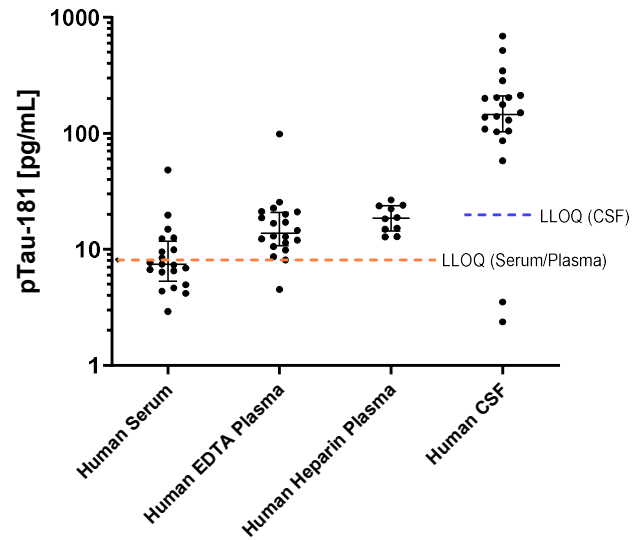
Kit Release Lower Limit of Quantification (LLOQ): The Kit Release LLOQ is a criterion applied during QC testing of each kit lot; it specifies the highest acceptable value for LLOQ. For QC testing, LLOQ is determined from HD-X or HD-1 data using the CV Profiling method described in the Technical Note, **Lower Limit of Quantification** (TECH-0034), available on the Quanterix Customer Portal. This is an analytical LLOQ and must be multiplied by the sample dilution factor to convert to functional LLOQ.

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 across 2 reagent lot and 2 instruments (3 runs per lot, per instrument).

Assay Range: The upper end of the dynamic range, or functional Upper Limit of Quantification (ULOQ) is equal to the minimum top calibrator concentration multiplied by MRD. Note that the concentration of the highest calibrator will vary between kit lots, as Reference Calibrators are value assigned to maintain consistency of results across lots.

Analytical LLOQ	2.00 pg/mL pooled CV 15.7% mean recovery 113.7%
Functional LLOQ (serum and plasma)	8.00 pg/mL
Functional ULOQ (serum and plasma)	1280 pg/mL
Functional LLOQ (CSF)	20.0 pg/mL
Functional ULOQ (CSF)	12,800 pg/mL
LOD	0.620 pg/mL range 0.084-1.211 pg/mL
Kit Release LLOQ	2.87 pg/mL

Endogenous Sample Reading: Presumably healthy donor matched human serum and EDTA plasma (n=20), and unmatched normal human heparin plasma (n=10) and CSF (n=20) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ for serum/plasma samples and blue line represents functional LLOQ for CSF samples.



Sample Type	Mean* pTau-181 pg/mL	Median pTau-181 pg/mL	% Above LOD	% Above LLOQ
Human Serum	17.0	7.42	100%	40%
Human Plasma	19.8	13.8	100%	95%
Human CSF	214	145	90%	90%
Human Heparin Plasma**	19.0	18.6	100%	100%

* Values below LLOQ are not included in the mean.

**For information only, not a fully validated matrix.

Precision: Measurements were obtained for 1 serum-based panel, 2 EDTA plasma-based panels, 1 CSF-based panel and 2 calibrator-based controls. Triplicate measurements were made for 12 runs (36 measurements in total) across 2 reagent lots and 2 instruments (3 runs per lot, per instrument). All samples were diluted at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	56.4	4.9%	4.7%	3.4%	3.7%
Control 2	622	3.0%	3.8%	1.7%	1.2%
Panel 1	23.9	7.0%	8.5%	10.2%	0.5%
Panel 2	23.5	6.7%	8.7%	9.9%	1.5%
Panel 3	664	3.0%	5.0%	2.9%	2.1%
Panel 4	238	2.4%	5.5%	5.1%	4.1%

Spike and Recovery: Spike recovery was measured with human serum and EDTA plasma samples in the feasibility phase of assay development. Results indicate that Matrix effects are observed with this assay, as a limited dilution was chosen to maximize the detectability / quantifiability of the analyte in serum and EDTA plasma samples from healthy donors.

Dilution Linearity: Dilution linearity was measured with human serum, EDTA plasma and CSF samples in the feasibility phase of assay development. Likely due to matrix effects, pTau-181 readings do not exhibit linearity upon dilution of the matrix with sample diluent. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

Admixture Linearity: Samples with high concentrations of analyte were created by spiking with calibrator. One EDTA plasma and one serum sample were mixed with low-analyte samples of the same matrix at 12 different ratios. The dilution series was run on the HD-X with the MRD (4x) dilution applied. Data was generated with two different lots of kits.

Admix Linearity Plasma low matrix 10.2 pg/mL high matrix 250 pg/mL	Mean 95% range 84–101% Slope = 0.9828, R ² = 0.9993
Admix Linearity Plasma low matrix 9.1 pg/mL high matrix 411 pg/mL	Mean 87% range 82–94% Slope = 0.9521, R ² = 0.9896
Admix Linearity Serum low matrix 6.58 pg/mL high matrix 242 pg/mL	Mean 93% range 83–100% Slope = 0.9894, R ² = 0.9983
Admix Linearity Plasma low matrix 5.9 pg/mL high matrix 96.7 pg/mL	Mean 114% range 66–117% Slope = 1.0262, R ² = 0.9935

Sample Freeze-Thaw Stability: Measurements were obtained with one endogenous EDTA plasma-based, one endogenous CSF-based panels and 2 EDTA plasma samples. Triplicate measurements were made for 3 Freeze-Thaw cycles on the same instrument using one lot of reagents. This data was generated using pTau-181 Advantage V2 kit. V2.1 kit uses the same antigen and antibodies as V2 kit.

Calibrator and Sample Stability: Measurements were obtained with 4 EDTA plasma samples and 7 calibrators. Triplicate measurements were made for 3 stability time points (3-hr and 7-hr stability at room temperature and 24-hr stability at 4 °C) on the same instrument using one lot of reagents. This data was generated using pTau-181 Advantage V2 kit. V2.1 kit uses the same antigen and antibodies as V2 kit.

Curve Storage: Measurements of one endogenous serum-based, one endogenous EDTA plasma-based, one spiked EDTA plasma-based, one endogenous CSF-based panels and 2 calibrator-based controls were obtained on HD-X. Triplicate measurements were made for 4 runs at 4 individual days across 8 days on the same instrument using one lot of reagents (4 runs total).

Drift: Measurements of one endogenous EDTA plasma-based, one spiked EDTA plasma-based and 2 calibrator-based controls were obtained on HD-X. Controls and panels were run across three plates with a total of 54 replicates per sample.

Sample Freeze-Thaw Stability (EDTA Plasma and CSF)	Mean 103% range 98.1–107%
Calibrator Stability	Mean 92.0% range 91.2–92.4%
Sample Stability	Mean 101% range 97.1–107%
Curve Storage	Mean Bias: 0.9% range 0.6–1.3%
Drift (Three-plate Variance)	Plate 1: 6.0% Plate 1+2: 6.5% Plate 1+2+3: 7.9%
Drift (Three-plate Precision)	Mean 6.8% range 0.2–13.0%

The Simoa pTau-181 Advantage V2.1 assay kit is formulated for use on the SR-X, HD-1, or HD-X platform. Some differences in performance claims between the HD and SR-X platforms may be observed when comparing data sheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples or may be due to minor differences in antibody and analyte behavior in the different assay formats.