Simoa® ALZpath p-Tau-217 Advantage PLUS Kit

HD-X Data Sheet Item 104570

Quanterix
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Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the ALZpath p-Tau 217 Advantage PLUS kit on the HD-X platform.

Threonine 217 is one of the phosphorylation sites of human tau protein (p-Tau 217). 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. In Alzheimer's disease (AD) and related neurodegenerative diseases, tau is abnormally phosphorylated and aggregated into bundles of filaments. The ALZpath p-Tau 217 Advantage PLUS Assay kit is a digital immunoassay for the quantitative determination of phosphorylated tau protein including p-Tau 217 in human EDTA plasma samples.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted in Figure 1. 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.

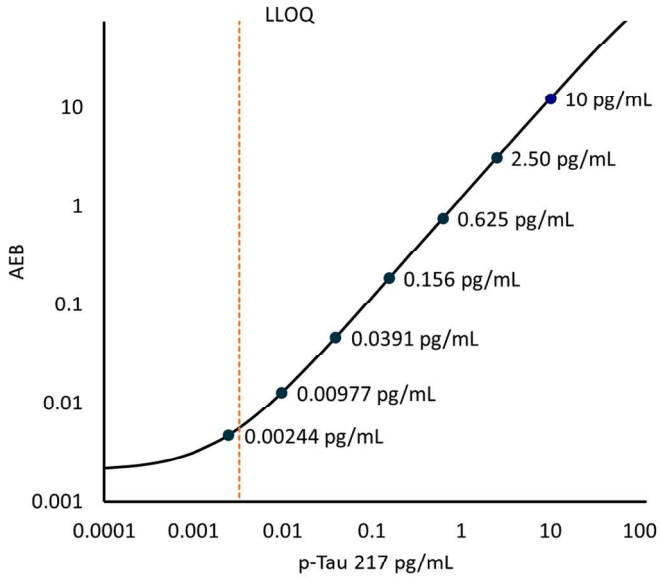

Figure 1. Example calibrator curve

Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
EDTA Plasma Dilution	1:3
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 each for 2 reagent lot across 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 (fLLOQ) values below are for plasma and represent the analytical LLOQ multiplied by the dilution factor used for the samples.

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 (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 top calibrator concentration multiplied by MRD.

Analytical LLOQ	**0.00326 pg/mL** pooled CV 17% mean recovery 112%
Functional LLOQ (3x MRD)	**0.00978 pg/mL**
Functional ULOQ (3x MRD)	**30 pg/mL**
LOD	**0.0008 pg/mL** range 0.0003–0.0021 pg/mL
Dynamic Range (3x MRD)	0 – 30 pg/mL

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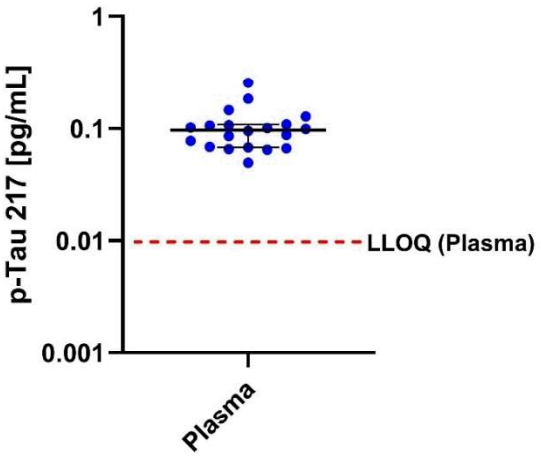
Endogenous Sample Reading: Healthy donor EDTA plasma (n=20) were measured. Bars depict median with interquartile range. Red line represents functional LLOQ.



Sample Type	Mean p-Tau 217 pg/mL	Median p-Tau 217 pg/mL	% Above LOD	% Above LLOQ
Plasma	0.103	0.097	100%	100%

Precision: Measurements of 2 plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 12 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	0.74	2.5%	8.1%	4.6%	2.8%
Control 2	7.56	3.1%	5.8%	2.0%	0.5%
Panel 1	0.14	5.3%	12.2%	5.4%	6.0%
Panel 2	9.49	5.1%	13.8%	3.5%	3.7%

Spike and Recovery: 2 EDTA plasma samples were spiked at high (5 pg/mL) and low (1 pg/mL) concentrations of peptide antigen, and at 5% volume of 2 CSF samples, and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of p-Tau 217 in the spiked sample and the measured concentration in unspiked sample relative to the concentration of p-Tau 217 in spiked calibrator diluent. 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 samples from healthy donors.

Dilution Linearity: 5 unspiked EDTA plasma samples were serially diluted with sample diluent through 3 levels of 2X dilutions. Each dilution series was run on the HD-X with the MRD (3x) dilution applied. Total dilution of each sample ranged from 3x to 24x. Due to matrix effects, p-Tau 217 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 identified. 2 EDTA plasma samples 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 (3x) dilution applied.

Spike and Recovery (Human EDTA plasma, peptide antigen spike)	**Mean: 22%** range 17–28%
Spike and Recovery (Human EDTA plasma, CSF spike)	**Mean: 50%** range 41–63%
Dilution Linearity (Human EDTA plasma, 3x - 24x)	**Mean: 125%** range 101–151%
Admixture Linearity Plasma (Human EDTA plasma)	**Mean: 95%** range 91–100%

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