LucentAD (Simoa p-Tau 181 LDT V2.1) is a novel, non-invasive, blood-based test for detection of tau protein phosphorylated at the threonine 181 site (pTau-181) using human plasma samples. This Single Molecule Array (Simoa®) immunoassay has been validated on the fully automated Quanterix HD-X analyzer as a Laboratory Developed Test (LDT) following CLSI guidelines. This LDT was developed as an aid in the diagnostic evaluation of Alzheimer's Disease (AD).

Description

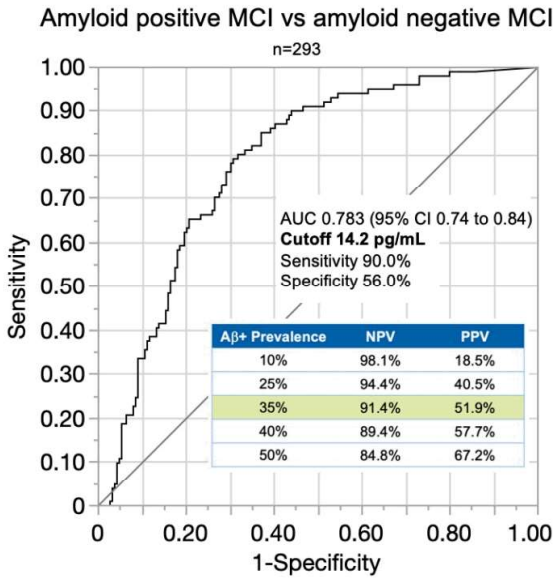
Threonine 181 is one of the phosphorylation sites of human tau protein (p-Tau 181).
Tau elevation is observed in the cerebrospinal fluid (CSF) of patients with neurodegenerative disease and severe head injuries, suggesting its extracellular release during neuronal damage and a role as a biomarker with 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. p-Tau 181 has been found to be more strongly associated with markers of AD than total tau. Quanterix's Simoa technology can measure clinically relevant changes in tau levels in plasma samples.
This datasheet summarizes data from the analytical validation performed at Quanterix to characterize the performance of the LucentAD p-Tau 181 Laboratory Developed Test on the Quanterix Simoa® HD-X platform.

LucentAD Test Performance

The LucentAD test was optimized to maximize clinical sensitivity and negative predictive value for patients with mild cognitive symptoms. A cohort of 293 subjects from multiple clinical sites in the U.S. who were diagnosed with MCI based on clinical and cognitive assessment was analyzed. Additionally, the brain amyloid status of each subject was determined by amyloid positron emission tomography (PET). Figure 1 depicts the performance of the test to detect amyloid pathology in this cohort of mildly impaired individuals.

A cutoff of 14.2 pg/mL yielded a clinical sensitivity of 90% and specificity of 56%. The inset table depicts negative and positive predictive values, NPV and PPV respectively, at varying prevalence of amyloid positivity. The prevalence of amyloid positivity in this validation cohort of 293 MCI patients was 34.5% (green shading). At this prevalence, the NPV of the test is 91.4%.

Figure 1



Aβ+ Prevalence	NPV	PPV
10%	98.1%	18.5%
25%	94.4%	40.5%
35%	91.4%	51.9%
40%	89.4%	57.7%
50%	84.8%	67.2%

Limit of Detection (LoD) and Lower Limit of Quantitation (LLoQ):

The LoD was calculated over 12 runs across 2 reagent lots and 2 instruments per CLSI EP-17. LoD was 0.2 pg/mL.

For assessment of LLoQ, a set of 6 EDTA samples were tested in 5 replicates per sample. Each of the 6 samples were selected to have a concentration at or near to the targeted LLoQ. The pooled %CV was calculated as %CV = 100 × SD_L /pooled mean. The functional LLoQ corresponding to the smallest concentration with a %CV not exceeding 20% was 9.1 pg/mL. The upper end of the measuring interval, Upper Limit of Quantification (ULoQ) is equal to the minimum top calibrator with the extended

measuring interval (EMI) equal to the ULoQ multiplied by minimum required dilution (MRD).

The reportable interval is shown below as described in CLSI EP34. All concentrations are pg/mL.

Linearity Assessment:
Two elevated EDTA plasma samples (antigen spiked) with values approximating the upper limit of the linearity interval (within 10%) were tested for admixture linearity (using 2 lots of reagents and 1 HD-X instrument) according to CLSI EP06. The samples were combined with an LLoQ level EDTA plasma samples across 10 dilutions. Linear regression was performed.

Summary: Deviation from linearity was ≤ 15% at all dilutions for the 4 admixture combinations tested (2 diluted sample series x 2 lots).

Parallelism:
Six elevated plasma samples with endogenous values between 10 and 60 pg/mL were serially diluted with sample diluent. Samples were run using a single lot of reagents according to CLSI EP34.

Summary: % Recovery against grand mean was calculated. The assay allows up to 8x dilution beyond ULoQ.

Imprecision Assessment:
Repeatability (intra-assay) and Reproducibility (inter-assay)
High, medium, and low plasma controls (EQCs), 5 native samples (antigen-supplemented) and 1 negative sample at LLoQ were diluted 10 times independently and tested in duplicate over 5 days, 2 runs per day using 1 reagent lot on 1 instrument (20 replicates per sample).

Sample/ Control Level	Repeatability (intra-assay)	Reproducibility (inter-assay)
Low	N/A*	15%
Medium	N/A*	9%
High	N/A*	3%
Sample 1	7%	15%
Sample 2	8%	15%
Sample 3	2%	14%
Sample 4	3%	12%
Sample 5	3%	7%
Sample 6 (LLoQ)	13%	11%

* Not assessed.

Summary: Intra-assay precision (repeatability) met the acceptance criteria of ≤ 15% CV. Inter-assay precision (reproducibility) met the acceptance criteria of ≤ 20% CV.

Inter-lot precision:
32 plasma samples (containing values in the moderate to upper end and the lower end of the measuring range) was performed in accordance with CLSI EP26. The samples were tested in duplicate with each of two reagent lots on the same HD-X instrument.

Mean Difference	11%
Average % CV	7.5%
95% CI for % CV	5.3% – 9.7%

Summary: The CI of the % difference was within the expected total imprecision of the assay. The grand mean % difference across all samples was observed to be no more than 11% between the two reagent lots.

Endogenous Interference:
Four EDTA plasma samples (high, mid, low, near LLOQ) were assessed for the impact of endogenous interferents on one lot of reagents (4 replicates per sample). Endogenous interferent spike concentration was based on CLSI EP-07 and EP-37.

Interferent	Concentration
Triglycerides	~1,000 mg/dL
Hemolysate	500 md/dL
Total protein (albumin)	12 g/dL
Bilirubin-conj	20 mg/dL
Bilirubin-unconj	20 mg/dL
Rheumatoid factor	~100 U/mL
Biotin	0.35 mg/dL
HAMA	~225 ng/mL

Summary: The table lists the highest level of interferent that recovered within 15% of target value.

Specificity / Cross-reactivity:
Tau peptides phosphorylated at four different amino acid residues (205, 217, 231 & 235) along with 181 (pos control) and unphosphorylated Tau 441 (neg control) were tested at 4 levels (near LLOQ to 10X LLOQ for p-Tau 181).

Summary: All cross-reactive peptides and unphosphorylated Tau 441 at all dilutions resulted in values below LLoQ.

Sample Requirements and Stability

Specimen	K2 EDTA Plasma
Minimum volume	0.5 mL
Collection container	Pearl-top gel barrier tube
Sample Stability	Refrigerated (2-8C): 7 days
	Frozen (-20C): 7 days
	Frozen (-80C): 14 days
Freeze/thaw stability	3 freeze/thaw cycles