LucentAD (Simoa p-Tau 217) is a novel, non-invasive, blood-based test for detection of tau protein phosphorylated at the threonine 217 site (pTau-217) 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 to help identify whether an individual with cognitive symptoms is likely or unlikely to have amyloid plaques in the brain, a hallmark of Alzheimer's disease.

Description

The LucentAD p-Tau 217 test measures tau protein phosphorylated at threonine 217. Circulating levels of p-Tau 217 have been shown to be a biomarker strongly associated with amyloid plaque pathology.[1,2] LucentAD p-Tau 217 is not a standalone diagnostic test. LucentAD results support a diagnostic assessment as an adjunct to other methods, such as clinical assessment, exclusionary blood workup, and cognitive evaluations. In uncertain cases, including an intermediate result from the LucentAD p-Tau 217 test, cerebrospinal fluid (CSF) biomarker tests or amyloid positron emission tomography (PET) may be indicated for further evaluation of amyloid pathology status to support a diagnosis.

The LucentAD p-Tau 217 validation performed at Quanterix on the Simoa® HD-X platform is summarized in this report.

LucentAD Test Performance

The LucentAD p-Tau 217 test was optimized to maximize clinical sensitivity and specificity for patients with cognitive symptoms. A 2-cutoff approach was utilized as recommended by the draft NIA-AA Revised Criteria for Diagnosis and Staging of Alzheimer's Disease[3] and Brum et al.[4] The use of two cutoffs establishes a three-zone test reflecting low, intermediate, and high risk of amyloid pathology. Samples reading at or below the lower cutoff are unlikely to have amyloid pathology, and samples reading at or above the upper cutoff are likely to have amyloid pathology. Test results in the intermediate range between the lower and upper cutoffs are considered to have an intermediate risk of amyloid pathology and may require referral for evaluation by other methods, including CSF biomarker testing.

The assay was validated with 873 subjects with known baseline amyloid beta status by CSF biomarkers or amyloid PET testing. Diagnostic categories ranged from mild cognitive impairment to Alzheimer's dementia. The overall test accuracy, defined as the ratio of correct results divided by the sum of correct results plus incorrect results as compared CSF biomarker test was 90.7%. Values from 0.041 and 0.089 pg/mL have increased uncertainty regarding amyloid pathology status. 30.9% of the validation samples gave results in the intermediate range.

Test Result p-Tau 217 (pg/mL)	Interpretation	Specificity / Sensitivity
≤ 0.040	Low likelihood of amyloid pathology	90.3% Sensitivity*
0.041 - 0.089	Intermediate likelihood of amyloid pathology	
≥ 0.090	High likelihood of amyloid pathology	91.3% Specificity*

*Excluding samples in the intermediate range.

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

The LoD was calculated over 2 runs across 2 reagent lots and 2 instruments per CLSI EP-17. LoD was 0.00129 pg/mL.

For assessment of LLoQ, 18 plasma LLOQ samples were tested in duplicate using 2 lots of reagents. The lowest analyte concentration that is reliably detectable within a specified target

range for bias (80-120%) and CV (≤20%) is reported as the LLOQ 0.003 pg/ml (functional LLOQ 0.006 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 as described by CLSI EP34 in in Figure 1. All concentrations are pg/mL.

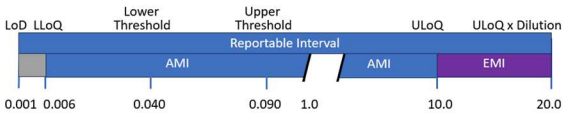

Figure 1. p-Tau 217 Reportable Interval

Linearity Assessment

Two elevated EDTA plasma samples (antigen spiked) with one sample with a value approximating the upper limit of the linearity interval (within 10%) and a second at ~ 5 pg/mL were tested for admixture linearity (using 1 lot 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 admixture combinations tested.

Parallelism

Five elevated plasma samples with endogenous values above the linear range 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 will allow up to 16x dilution beyond ULoQ.

Imprecision Assessment

Repeatability (intra-assay) and Reproducibility (inter-assay)

Low and high plasma controls (EQCs), 5 native samples and 1 sample near LLoQ were diluted 10 times independently and tested in duplicate over 5 days, 2 runs per day using 2 reagent lots on 2 HD-X instruments. All samples were tested in duplicate to obtain 20 replicates per sample per instrument based on EP05.

Sample/ Control Level	Repeatability (intra-assay)	Reproducibility (inter-assay)
Low	N/A*	16%
High	N/A*	4%
Sample 1	11%	11%
Sample 2	8%	10%
Sample 3	6%	9%
Sample 4	5%	9%
Sample 5	8%	16%
Sample 6 (LLoQ)	13%	18%

* Not assessed.

Inter-lot precision

30 endogenous plasma samples with values bracketing the medically relevant range were evaluated for lot-to-lot imprecision in accordance with CLSI EP26. The samples were tested in duplicate with each of 2 reagent lots on the same HD-X instrument.

Mean Difference	8%
Average % CV	6.1%
95% CI for % CV	4.6% – 7.6%

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 8% between the two reagent lots.

Endogenous Interference

Three EDTA plasma samples (high, mid, and low) were assessed for the impact of endogenous interferents on one lot of reagents in duplicate for each sample. Endogenous interferent spike concentration was based on CLSI EP-07 and EP-37.

Interferent	Concentration
Triglycerides	1,000 mg/dL
Hemolysate	500 mg/dL
Total protein (albumin)	1 g/dL
Bilirubin-conjugated	20 mg/dL
Bilirubin-unconjugated	20 mg/dL
Rheumatoid factor	95 U/mL
Biotin	0.360 mg/dL
HAMA	10 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 different amino acid residues (181, 205, 212, 231 & 235) along with 217 (pos control) were tested at 4 levels (0.03, 0.3, 3 and 30 pg/mL of each spike peptide).

Summary All cross-reactive peptides up to 3 pg/mL dilutions resulted in cross reactivity ≤15%. p-Tau 212 showed expected cross-reactivity with the assay at 30 pg/mL.

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): 14 days
	Ambient: 8 hours
Freeze/thaw stability	3 freeze/thaw cycles

About the LucentAD Test

LucentAD test results should only be used in conjunction with other clinical information when evaluating patients. This test was developed and its performance characteristics determined by Quanterix. It has not been cleared or approved by the US Food and Drug Administration.

References

1. Therriault J, Vermeiren M, Servaes S, et al. Association of Phosphorylated Tau Biomarkers With Amyloid Positron Emission Tomography vs Tau Positron Emission Tomography. JAMA Neurol. 2023;80(2):188-199.
2. Doré V, Doecke JD, Saad ZS, et al. Plasma p217+tau versus NAV4694 amyloid and MK6240 tau PET across the Alzheimer's continuum. Alzheimer's Dement (Amst). 2022;14(1):e12307. Published 2022 Apr 5.
3. NIA-AA Revised Criteria for Diagnosis and Staging of Alzheimer's Disease. Draft Oct 9, alz.org/NIA-AA.
4. Brum WS, Cullen NC, Janelidze S, et al. A two-step workflow based on plasma p-tau217 to screen for amyloid β positivity with further confirmatory testing only in uncertain cases. Nat Aging. 2023;3(9):1079-1090.