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More Results for More Patients

LucentAD Complete: A Multi-Analyte Algorithmic Blood Test to Aid in the Diagnostic Evaluation for Alzheimer's Disease

Introduction

The emergence of new Alzheimer's therapies has elevated an urgent need for improved tools to help diagnose the disease in its early stages, when therapeutic intervention is most likely to deliver clinical benefit. While Alzheimer's disease (AD) diagnostics has historically relied on clinical, symptom-based diagnosis, biomarkers are clearly playing an increasingly important role. Both amyloid and phosphorylated tau have been shown in many clinical studies to be highly predictive biomarkers of the presence of amyloid pathology, the hallmark of AD. Until recently, measurement of these biomarkers has required positron emission tomography (PET) or cerebrospinal spinal fluid (CSF), modalities that are expensive and/or invasive. Fortunately, in tandem with progress in Alzheimer's therapeutics, there has been rapid progress on the development of blood-based biomarker tests that have the potential to enable non-invasive alternatives for assessing amyloid pathology status. Blood-based biomarkers hold the promise of expanding access to biomarker testing by reducing the reliance on expensive and invasive procedures and facilitating more efficient and accurate diagnosis.

Among blood-based biomarkers, tau phosphorylated at amino acid 217 (p-Tau 217) measured in plasma is now considered the most accurate single biomarker for detecting amyloid pathology. Reflecting this consensus, the Alzheimer's Association (AA) Workgroup has recommended plasma p-Tau 217 as the only blood-based biomarker that has demonstrated accuracy comparable to FDA-cleared CSF biomarker tests, enabling a confirmatory diagnostic use case.[1] The AA criteria also recommends that a blood test for plasma p-Tau 217 be designed with two cut-offs in recognition of signal overlap between diseased and non-diseased patients, a recommendation shared by others.[2] The use of two cutoffs maximizes the negative and positive predictive values of the test but also results in a diagnostic 'gray zone' of intermediate risk in which there is uncertainty of the amyloid status and an inconclusive result. The AA further recommended the plasma test should exhibit an accuracy for amyloid classification of ≥90% for diagnostic use. Aligned with this, the Us Against Alzheimer's Global CEO initiative (CEOi)[3] similarly arrived at recommendations on confirmatory diagnostic plasma test performance criteria and a 2-cutoff approach that mirrors that of the AA.[4] In addition, the CEOi workgroup recommendations suggest that in order to benefit the greatest proportion of the intended use population, the intermediate zone of the test should be at or less than about 20% of all patients tested.[4] A key challenge is accurately classifying the amyloid status of borderline cases falling in the intermediate zone.

Along with p-Tau 217, several other plasma biomarkers are relevant in detecting the presence of amyloid pathology, either reflecting amyloid pathology directly or by detecting the presence of AD-associated disease pathways. The non-tau biomarkers that have received the greatest attention are amyloid $ß_{1-40}$ (Aß40), amyloid $ß_{1-42}$ (Aß42), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL). The plasma amyloid biomarkers, typically measured as a ratio of Aß42/Aß40 to improve the signal, directly reflect the status of amyloid plaque development.[5] Plasma GFAP reflects astrocytic cell activation in the brain, which occurs in concert with amyloid pathogenesis.[6] NfL is a general

biomarker of brain neuroaxonal damage that occurs in neurodegenerative diseases, including AD.[7] Each of these non-tau plasma biomarkers have been found to be predictive of the presence of AD, and the combination these biomarkers has improved the predictive power of a blood test relative to each biomarker alone.[8] However, of the combination of these biomarkers remains less accurate than p-Tau 217 alone.[9] In addition, a predictive model of combining p-Tau 217, GFAP and NfL has been found to be no better than that of p-Tau 217 alone.[10]

While the non-tau biomarkers have not been found to be particularly beneficial in combination with p-Tau 217 for amyloid classification of non-borderline cases, Quanterix discovered that the logistic combination of these biomarkers benefited accurate amyloid classification of borderline intermediate cases from p-Tau 217 alone.[11] This multi-marker algorithm substantially reduces the intermediate zone (by almost 3-fold in validation studies) while maintaining high overall accuracy. Thus, the test combines the predictive power of p-Tau 217 for cases with low and high amyloid burden, with additional amyloid classification power for borderline cases through the addition of these other four AD-relevant biomarkers. Quanterix has combined these four augmentative biomarkers into a single multiplexed test to complement the p-Tau 217 test to provide accurate amyloid classification results for more patients. The two tests together have been developed and clinically validated as a laboratory developed test (LDT) branded "LucentAD Complete" for clinical use to provide accurate results to the greatest number of patients with a simple, scalable immunoassay-based format.

How LucentAD Complete works

LucentAD Complete utilizes the LucentAD p-Tau 217 assay[12,13] and the Simoa N4PE multiplex (Aß42/Aß40, GFAP*, and NfL) for a more complete interrogation of plasma samples for amyloid pathology than can be provided by p-Tau 217 alone. The test relies on quantitation of p-Tau 217 for amyloid classification of patients with low or high amyloid burden, and the combination of p-Tau 217 and the four additional AD-relevant biomarkers in a multi-variant logistic algorithm to enhance discernment of amyloid status for most intermediate borderline cases. The logistic results are converted to an amyloid risk score with a range of 0 to 100. The risk score is compared with lower and upper cutoffs to establish the amyloid classification as either low, intermediate, or high risk. The intermediate range of the algorithmic scale represented approximately 11-12% of all samples tested in the validation cohorts summarized in the section below. Thus, conclusive results (≥90% certainty) were obtained for nearly 90% of intended-use patients tested. **Figure 1** below illustrates how the test works.

Figure 1: Multi-analyte Interrogation of Plasm Samples for Amyloid Status

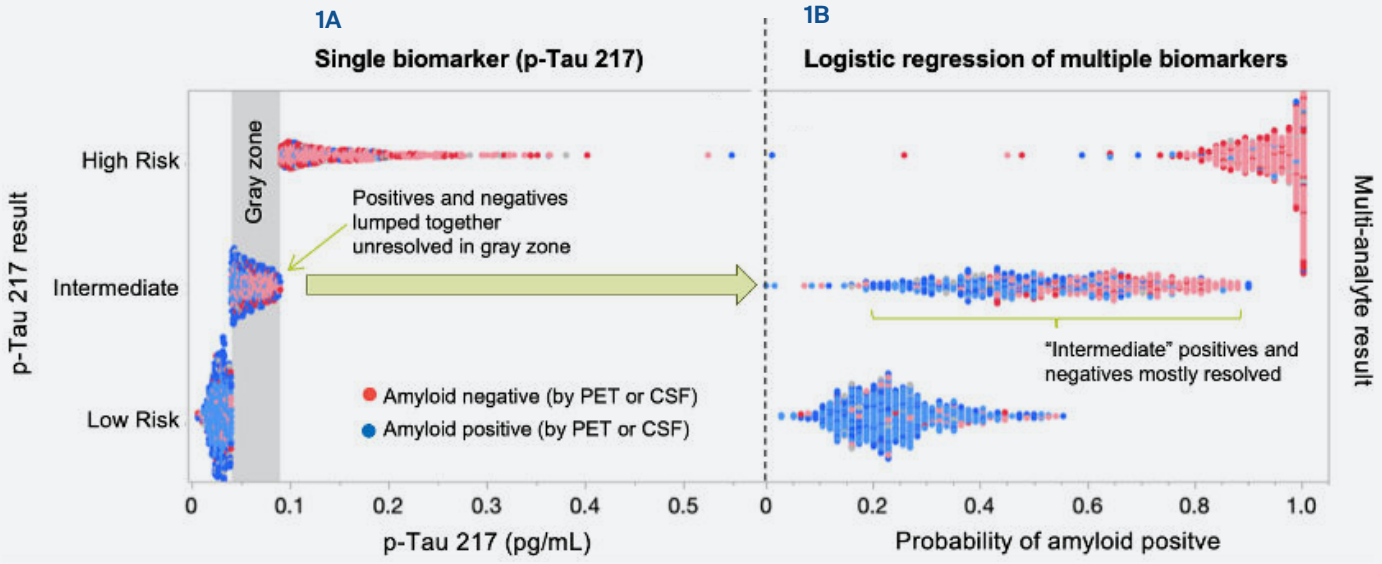

Fig 1. LucentAD Complete utilizes p-Tau 217 together with Aß42/Aß40, GFAP, and NfL to accurately classify the amyloid status in symptomatic patients. Inclusion of additional analytes using multi-variate logistic regression improves upon single-analyte performance (Fig 1A) providing accurate amyloid classification for a larger percentage of patients (Fig 1B). All results are converted to an amyloid risk score for test readout and interpretation.

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In a highly diverse validation cohort of 545 patients (described below), the multi-analyte interrogation accurately classified the amyloid status in 123 out of 185 p-Tau 217 intermediate cases, reducing the number of inconclusive results by two-thirds. This was a 3-fold reduction in the intermediate zone from 33.9% to 11.4%.

Analytical Performance of the LucentAD Complete Test

Sensitivity The analytical sensitivity of LucentAD Complete is dictated by the analyte of lowest physiological concentration in the plasma of cognitively normal and impaired individuals. p-Tau 217 is a lower abundance protein in plasma than Aß40, Aß42, GFAP, or NfL, and it is difficult to reliably measure this analyte in all individuals with conventional analog immunoassay or mass spectrometry. The digital p-Tau 217 assay used in LucentAD Complete has a limit of detection of 0.0015 pg/mL, and a functional limit of quantification of 0.006 pg/mL.[13] With this level of sensitivity, p-Tau 217 has been reliably measured by LucentAD p-Tau 217 and LucentAD Complete in 100% of over 2,000 clinical samples tested to date, ranging from cognitively normal to Alzheimer's dementia.

Precision With a multi-analyte algorithmic test, the precision of the logistic risk score is dependent upon the precision of all the assays in the test. LucentAD Complete measures five different analytes. The precision of four of the five tests (Aß40, Aß42, GFAP, and NfL) is largely dictated by the precision of a single multiplexed immunoassay in which all assays are performed simultaneously, thus reducing the variability of four different immunoassays. This consolidation of multiple tests into a single assay benefits the overall precision of the algorithm. To assess the expected precision of LucentAD Complete, the imprecision of each of the five assays was characterized individually in a multi-day study, and assay variances were randomly added to or subtracted from each of 1,305 clinical samples. 10 simulations were performed for each of the 1,305 samples (to mimic a precision study of 10 runs/sample), and the imprecision of the risk score output and its effect on amyloid classification accuracy was evaluated. The overall mean imprecision (%CV) of the test across all simulations was 7.33% (95% CI: 6.87-7.80%). At this level of precision relative to clinical cutoffs, the potential low to high or high to low amyloid risk misclassification was estimated to be 1%.

Clinical Performance of the LucentAD Complete Test

To establish the clinical performance of the LucentAD p-Tau 217 test, EDTA plasma samples were tested in duplicate in the Quanterix CLIA laboratory. The clinical sample sets included patients with MCI and AD dementia from three independent cohorts: the Amsterdam Dementia Cohort (ADC),[14,15] recruited individuals with MCI and mild AD from the prospective BioHermes trial,[16] and individuals from a longitudinal cohort from ADNI.[17] The ADC represents a memory clinic setting with CSF biomarkers as the method for determining amyloid status. The BioHermes trial included recruited participants from 17 US sites with emphasis on racial/ethnic diversity and utilized amyloid PET to determine amyloid status. 27.8% of the impaired participants in the BioHermes cohort were from under-represented racial/ethnic groups, including White Hispanics.[16] The ADNI cohort included participants from multiple North American memory centers and utilized amyloid PET to determine amyloid status. Demographic characteristics of the cohorts are shown in **Table 1**.

Table 1: Demographic Characteristics of Clinical Cohorts

	ADC cohort 1	ADC cohort 2	BioHermes training sub-cohort	BioHermes validation sub-cohort	ADNI longitudinal cohort
n	495	274	235	271	537
Mean age	65 yr	65 yr	73 yr	73 yr	70 yr
Proportion female	48%	47%	52%	55%	35%
APOE ε4 allele carriership	61%	61%	42%	40%	Unk
White, incl White Hispanic	91%	91%	85%	85%	94%
Aβ positive	81%	71%	48%	45%	53%
MCI	45%	65%	58%	55%	83%
Mild AD/AD	55%	35%	42%	45%	17%

Table 1. Partial demographic characteristics of three clinical cohorts used for training and validation of LucentAD Complete. The ADNI cohort represents 537 samples from 173 individuals. The Aß+ prevalence for this cohort reflects increasing prevalence across a maximum of 15 years on a diminishing number of participating subjects.

ADC = Amsterdam Dementia Cohort
MCI = mild cognitive impairment
AD = Alzheimer's disease
Unk = APOE e4

(allele data were not available at the time of this document.)

Training and thresholds for the multi-analyte algorithm (risk score 0.45 and 0.70) utilized the ADC cohort 1 (n = 495) and the BioHermes training sub-cohort (n = 235). The multi-analyte thresholds were validated with ADC cohort 2 (n = 274) combined with the BioHermes validation sub-cohort (total n = 271), and the ADNI longitudinal cohort (n = 537). **Table 2** summarizes the clinical performance metrics obtained, while **Figure 2** provides a visual representation of the distribution of LucentAD Complete risk score results across the validation cohorts.

Table 2: Clinical Performance of LucentAD Complete

Table 2. Clinical performance parameters of Lucent AD Complete LDT. *Note: performance parameters exclude samples in the intermediate zone.*

Table 2A. Performance statistics broken out by training and validation cohorts (validation cohorts separated and combined).

Table 2B. Test performance for a subset of ADNI participants with aligned timepoints illustrating performance reproducibility in smaller longitudinal samplings. These results, on the same individuals repeatedly sampled over 4 years, illustrate the performance levels and apparent variation expected in smaller sampling sizes.

2A

	Cross sectional		Longitudinal	Combined
Cohorts:	Training	Validation	Validation	Validation
n	730	545	537	1,082
AUC	92%	91%	92%	92%
Prevalence	70%	58%	53%	55%
False neg rate	9%	10%	9%	9%
False pos rate	8%	9%	9%	9%
% in Intermediate	11%	11%	13%	12%
Accuracy	90%	89%	90%	90%
Sensitivity	90%	88%	91%	90%
Specificity	90%	90%	89%	90%
PPV	96%	93%	91%	92%
NPV	78%	85%	89%	87%

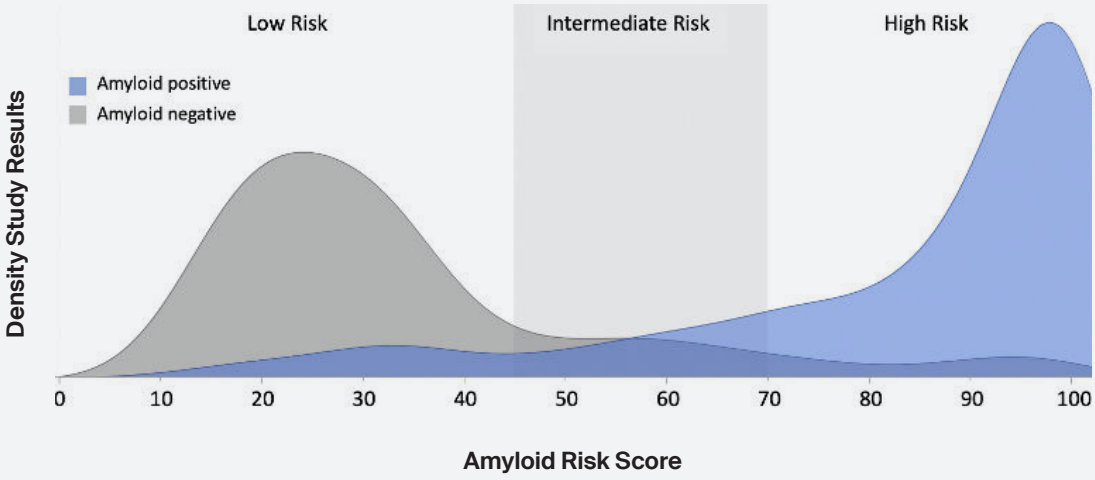
2B

ADNI, single sample/individual		
Baseline	24 mo	48 mo
115	107	111
94%	94%	88%
46%	47%	51%
11%	10%	14%
7%	5%	13%
11%	8%	14%
90%	92%	84%
88%	89%	84%
91%	94%	85%
90%	93%	86%
89%	91%	83%

AUC = area under the curve **PPV** = positive predictive value **NPV** = negative predictive value

Figure 2: Density of Validation Results

Fig 2. Density plot of LucentAD Complete risk scores across the validation cohorts (n = 1,082). 88.1% of the results gave amyloid classification results outside the intermediate zone. Comparator amyloid status was from amyloid PET or CSF biomarkers.



The results in **Table 2** demonstrate robust and reproducible clinical performance across three highly diverse cohorts and amyloid positivity prevalences. The diversity included differences in race/ethnicity, age, geographies, clinical settings and comparator methods. The positive predictive value (PPV) remained >90% across amyloid prevalences ranging from 46% to 70%.The combined validation statistics met target accuracy standards of 90%,[1,4] and the intermediate range (11.9%) was well below the suggested maximum of 20%.[4] **Figure 2** shows good separation between amyloid negative and positive subjects as determined by CSF or amyloid PET.

Using and Interpreting the LucentAD Complete Test

The LucentAD Complete test is intended to be used in patients with objective evidence of cognitive impairment who are being evaluated for AD to aid in diagnostic evaluation. Objective evidence may be from a cognitive test. LucentAD Complete reports an amyloid risk score from 0 to 100. A low amyloid risk below 45 indicates a low likelihood of the presence of amyloid pathology. Alternative causes for the patient's memory concerns should be investigated. An elevated amyloid risk score above 70 indicates a high likelihood of the presence of amyloid pathology. An elevated result above 70 is consistent with the presence of Alzheimer's disease but does not in itself establish a diagnosis. Test results in the intermediate range between 45 and 70 are associated with an intermediate likelihood of amyloid pathology and less certainty of amyloid status. If clinically indicated, an intermediate result may require referral for evaluation by other methods such as CSF biomarker testing or PET imaging, or a return blood test at a later time. **Table 3** summarizes the interpretation of the LucentAD Complete test results.

Table 3: LucentAD Complete Test Result Interpretation

Amyloid Risk Score	Test Result Interpretation
<45	**Low likelihood** of amyloid pathology.
45–70	**Intermediate likelihood** of amyloid pathology.
>70	**High likelihood** of amyloid pathology.

LucentAD Complete is not a standalone diagnostic test. LucentAD Complete 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 Complete test, CSF biomarker tests or amyloid positron emission tomography (PET) may be indicated for further evaluation of amyloid pathology status to support a diagnosis.

Summary of LucentAD Complete Test and Intended Use Population

The LucentAD Complete is a multi-analyte logarithmic blood test that helps identify whether an individual with objective cognitive symptoms is likely or unlikely to have amyloid plaques in the brain, a hallmark of AD. The test relies on quantitation of p-Tau 217 in plasma to accurately classify the amyloid status of most impaired patients with low or high amyloid burden, and quantitation of additional AD-relevant biomarkers (Aß42/Aß40, GFAP, and NfL) to enhance discernment of amyloid status for most intermediate borderline cases. The test uses proprietary single molecule array (Simoa) technology[18] and the fully automated HD-X instrument[19] to provide unprecedented analytical sensitivity and precision for measuring low abundance proteins. This level of sensitivity and precision provides a best-in-class ability to measure p-Tau 217 concentration in **every clinical sample**, and a highly precise algorithmic risk score output. Simoa multiplexing enables simultaneous measurement of Aß42/Aß40, GFAP, and NfL in a single, precise assay.

LucentAD Complete is an ultrasensitive fully automated multi-analyte digital immunoassay that does not require time-intensive nucleic acid amplification steps, sample enrichment, or complex mass spectrometry procedures. LucentAD Complete is a scalable readily accessible blood test for a more fully informed evaluation of patients as an aid toward a diagnosis. The test also provides individual biomarker results for all AD-relevant biomarkers, including NfL which can be separately interpreted as a marker of non-AD neurodegenerative pathology, such as frontal temporal dementia.*

**Available in a future product update.*

Summary

LucentAD Complete meets or exceeds current recommendations for test performance required in a blood-based diagnostic for amyloid pathology.[1,3] The test provides a blood-based method for accurate amyloid risk classification in patients exhibiting objective cognitive symptoms as an aid in diagnostic evaluation.

Ordering Information

To order LucentAD Complete collection materials go to **LucentDiagnostics.com**

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