

Comparison of a Multi-Analyte Algorithmic Immunoassay Blood Test with Immunoprecipitation Mass Spectrometry for the Detection of Amyloid Pathology*

David Wilson,^{1*} Karen Copeland,² Lindsey Mette,¹ Meenakshi Khare,¹ Jorge Marques Signes,¹ Tim Skelton³

¹Quanterix Corporation, 900 Middlesex Turnpike, Billerica, MA, 01821, USA; ²Boulder Statistics, 760 Twilight Ln Steamboat Springs, CO, 80487, USA;

³Lahey Hospital & Medical Center, Burlington, MA, United States 01813, USA *Presenting/corresponding author

Contact: dwilson@quanterix.com



Lucent Diagnostics



*Originally accepted as abstract: "Comparative Performance Analysis of a Multi-Analyte Algorithmic Immunoassay in a Defined Cross-Sectional and Longitudinal ADNI Cohort"

Background

Alzheimer's blood-based biomarker tests using p-Tau 217 or p-Tau 217/Aβ42 ratios are well-characterized, but head-to-head comparisons of multi-analyte algorithmic tests on shared cohorts are lacking. We compared a 5-biomarker algorithmic immunoassay (**LucentAD[®]Complete**) with an algorithmic immunoprecipitation mass-spectrometry, IP-MS (PrecivityAD2™) benchmark that utilizes %p-Tau 217 and Aβ42/40 ratios on a shared plasma cohort.

Methods

To benchmark against previously published IP-MS data [1], we matched the IP-MS comparator's cohort (**n=191 unique symptomatic participants**) using baseline and longitudinal samples from the same FNIH/ADNI dataset. Of the original 355 participants provided by FNIH [2], 176 cognitively normal individuals were excluded, leaving **192 unique symptomatic participants** (with MCI or dementia). Performance was assessed across a cross-sectional sub-cohort (n=115) with available baseline data and a longitudinal sub-cohort (n=179 samples). Demographic data are provided in **Table 1**.

The longitudinal group included follow-up visits from 102 baseline individuals plus 77 subjects with follow-up data only, providing a broad representation of clinical latency toward dementia (**12–144-month span**). This reflects the heterogeneous disease stages and higher amyloid prevalence encountered in real-world practice. Unlike cross-sectional studies—which can reflect an idealized, narrow window of early disease—this longitudinal distribution captures the late-stage pathology and clinical 'noise' characteristic of specialist evaluations. These later timepoints provide a more rigorous test of diagnostic stability than simple baseline designs. To maintain stringency, samples were excluded if a participant's clinical classification reverted to cognitively normal; this resulted in the exclusion of 13 longitudinal samples. All samples were analyzed within six months of the closest amyloid PET scan. [2].

Assay, Aβ PET Status, and Analysis. Amyloid PET imaging utilized standard protocols for Florbetapir or Florbetaben, with the Centiloid (CL) unit estimate applied by the ADNI PET Core [3]. Amyloid positivity was defined as > 20 centiloids, per FNIH study [2]. IP-MS data from the PrecivityAD2™ were reported previously [1]. The multi-analyte immunoassay leverages the **four-axis, ATN(I) pathophysiological framework** [4,5] representing four distinct biological disease axes (**Figure 1**). These include p-Tau 217 ("T": LucentAD p-Tau 217, a Janssen assay design [6]), amyloid β-42/40 ratio ("A": Aβ42/40), neurofilament light chain ("N": NfL), and glial fibrillary acidic protein ("I": GFAP). The latter four biomarkers are quantified utilizing the multiplexed N4PE kit. The LucentAD Complete algorithm integrates these biomarkers via a logistic regression to generate a unified amyloid Risk Score. Development and clinical validation of LucentAD p-Tau 217 and LucentAD Complete are described elsewhere [6, 7].

Figure 1: LucentAD Complete: Four-Axis "ATN(I)" Plasma Test

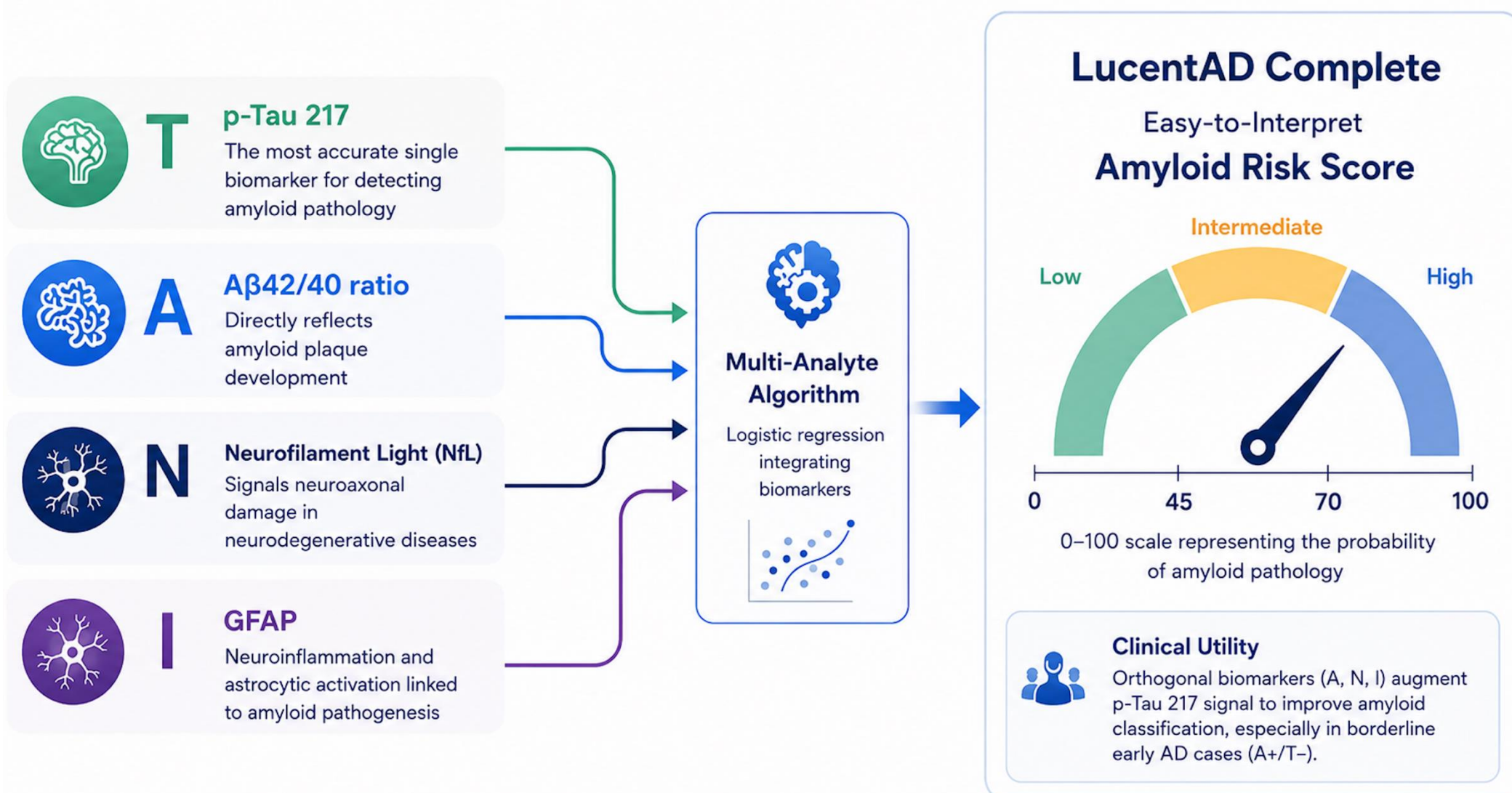


Figure 1. LucentAD Complete integrates four key Alzheimer's-relevant biomarkers representing the **ATN(I) framework** (Jack *et al*¹, Imbimbo *et al*⁵) into a composite Amyloid Risk Score. p-Tau 217 is the backbone of the test, and the orthogonal biomarkers Aβ42/40 ratio, NFL, and GFAP augment the p-Tau 217 signal for amyloid classification in difficult borderline early AD cases (amyloid+, tau-, A+/T-). **The LucentAD Complete test report includes the Amyloid Risk Score and the individual ATN(I) biomarkers values alongside interpretive normative ranges for additional potential clinical insights.**

LucentAD Complete generally resolves ~2/3's of borderline cases that cannot be classified by p-Tau 217 alone. This additional resolution of borderline intermediate cases is illustrated in **Figure 2**.

Figure 2: LucentAD Complete - Resolution of Intermediate Borderline Cases

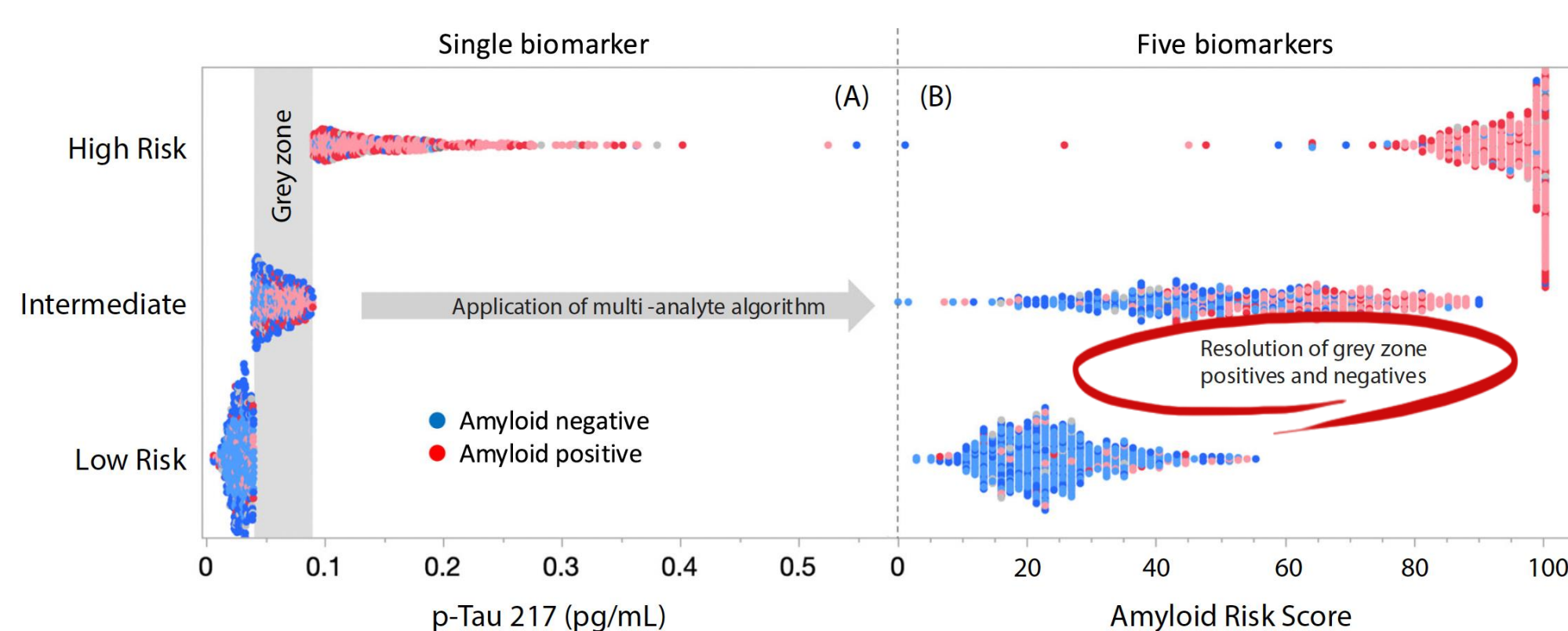


Figure 2. Multi-analyte interintegration of plasma samples for amyloid status. **A.** p-Tau 217 classifies samples with low and high amyloid burden, identifying intermediate risk cases. **B.** Multivariate analysis with all four of the ATN(I) biomarkers resolves majority of intermediate cases. All results are converted to an Amyloid Risk Score for final interpretation.

Results

Table 1: Demographic & Clinical Characteristics of the Symptomatic ADNI Cohort

Characteristic	Baseline Sub-cohort (n=115)	Longitudinal Sub-cohort (n=179)
Age, mean (SD)	69.6 (7.2)	70.7 (7.0)
Sex (Female), n (%)	53 (46.1%)	81 (45.3%)
Race/Ethnicity		
White, n (%)	108 (93.9%)	168 (93.9%)
Non-White, n (%)	7 (6.1%)	11 (6.1%)
Hispanic or Latino, n (%)	7 (6.1%)	8 (4.5%)
Clinical Diagnosis		
MCI, n (%)	113 (98.3%)	151 (84.4%)
Dementia, n (%)	2 (1.7%)	28 (15.6%)
Amyloid Status (PET)		
Positive, n (%)	47 (40.9%)	97 (54.2%)
Negative, n (%)	68 (59.1%)	82 (45.8%)

Table 1. Baseline characteristics for the **unique symptomatic participants (N=192)** and corresponding cross-sectional (n=115) and longitudinal (n=179) sub-cohorts. Note the increase in dementia and amyloid prevalence from baseline to the final longitudinal follow-up (shaded), illustrating the disease progression captured by the study design. 13 longitudinal samples were excluded because the participant's clinical status reverted to cognitively normal at the time of draw, ensuring the analysis focused strictly on the symptomatic disease trajectory.

Figure 3: Amyloid Discrimination by Individual Biomarker and LucentAD Risk Score

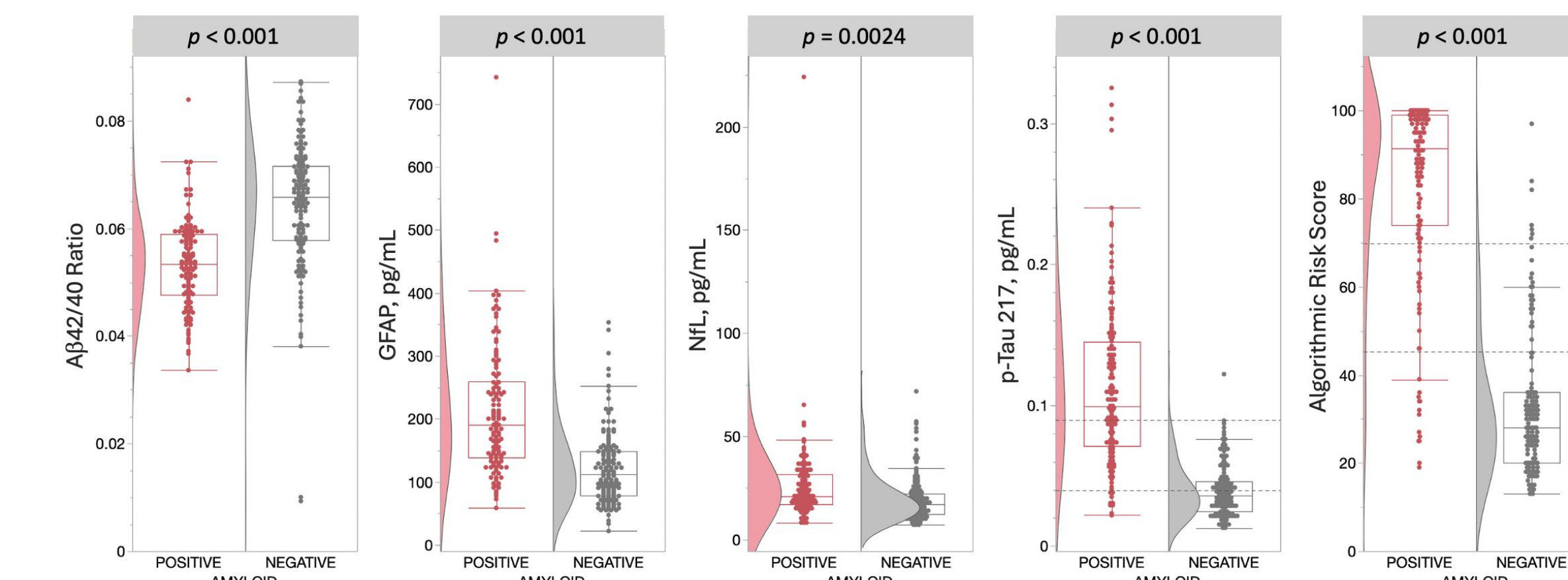


Figure 3. Distribution of plasma biomarker concentrations by amyloid status. Boxes represent the upper and lower quartiles, the horizontal line in the boxes represents the median value, and the whiskers extend to 1.5 times the interquartile range. These individual biomarker signals provide the biological foundation for the multi-analyte algorithmic Risk Score. Horizontal dashed lines depict pre-specified validated lower and upper clinical cutoffs for p-Tau 217 [10] and LucentAD Complete [2].

Figure 4: Standalone p-Tau 217 vs. LucentAD Complete Multi-Analyte Algorithm

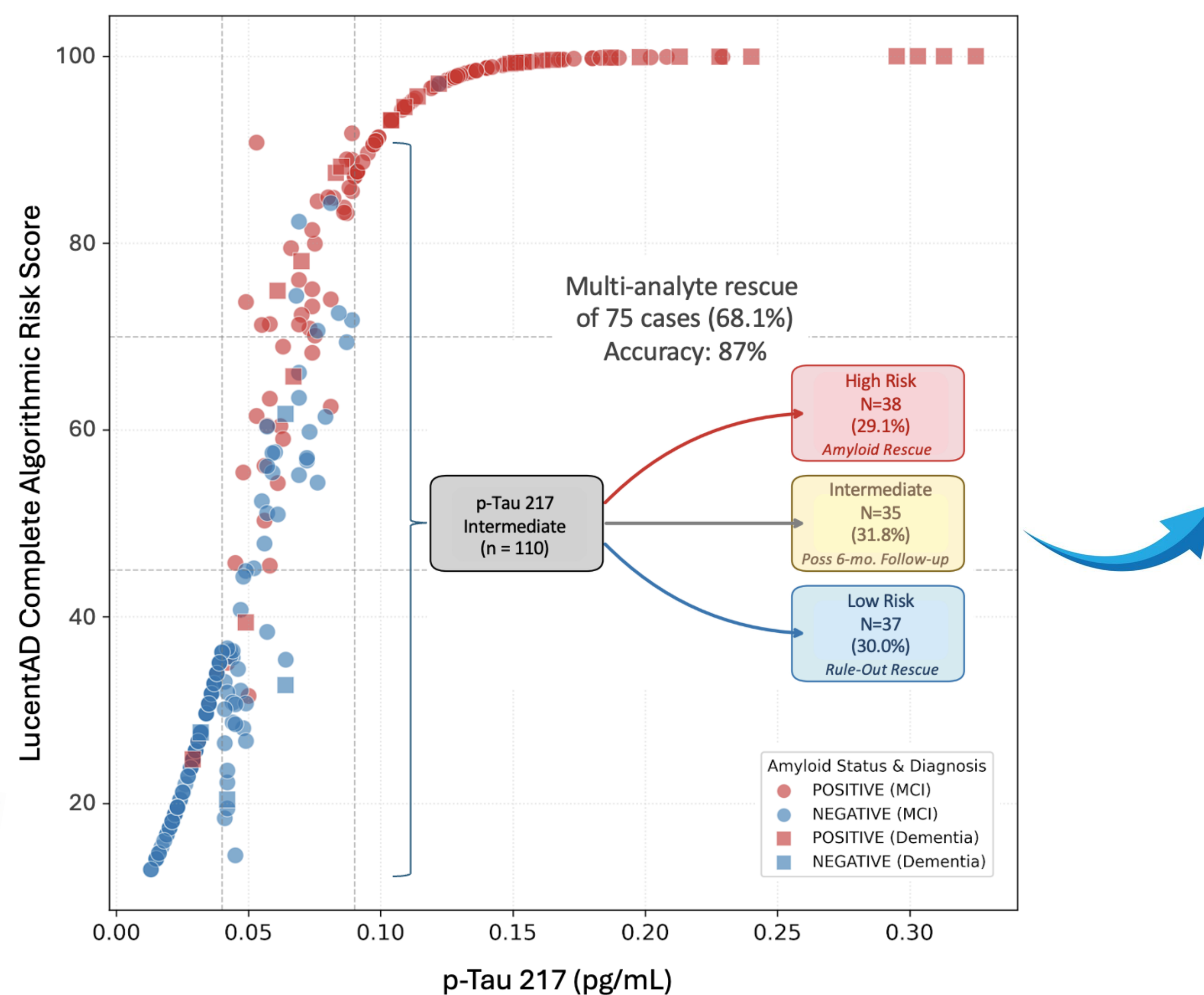


Figure 4. Algorithmic resolution of diagnostic uncertainty in the p-Tau 217 intermediate zone. Concordance between standalone p-Tau 217 and the multi-analyte Risk Score across the clinical spectrum (n=294). Dashed lines indicate lower and upper cutoffs, **0.04–0.09 pg/mL** for p-Tau 217 assay, and **45–70** for the multi-analyte assay. Note that the multi-analyte algorithm is only applied to intermediate cases identified by p-Tau 217. This maintains assay specificity against non-AD elevations of NFL and GFAP. The influence of the secondary biomarkers on the Risk Score at the p-Tau 217 cutoff transitions is evident, especially at the lower cutoff.

Results (cont.)

Table 2: Diagnostic Performance of LucentAD Complete vs. IP-Mass Spectrometry

Performance Measure	Mass Spectrometry Benchmark ^a (n=191)	LucentAD Complete Baseline (n=115)	LucentAD Complete Longitudinal ^c (n=179)	LucentAD Complete Aggregate ^c (n=294)
Aβ Prevalence	56.0%	40.9%	54.2%	49.0%
AUC	0.95	0.94 (0.88–0.97)	0.94 (0.89–0.97)	0.94 (0.91–0.97)
Accuracy ^d	91%	93.2%	91.7%	92.3%
Sensitivity ^d	90%	90.0%	89.9%	89.9%
Specificity ^d	92%	95.2%	94.0%	94.6%
False Negative Rate	10% ^b	8.5%	9.3%	9.0%
False Positive Rate	8% ^b	4.4%	4.9%	4.7%
PPV	93%	92.3%	95.2%	94.3%
NPV	88%	93.8%	87.5%	90.4%
Intermediate Zone	N/A	10.4%	12.8%	11.9%

Table 2. ^aPrecivityAD2 metrics as reported in [6]. Intermediate zone is "N/A" because a single-cutoff binary classification was used. ^bFalse negative and false positive rates were derived from reported sensitivity and specificity values. ^cCohort composition: The longitudinal sub-cohort includes follow-up visits from 102 baseline subjects plus 77 subjects with follow-up data only. ^dAccuracy, Sensitivity, Specificity, PPV and NPV are calculated excluding the intermediate zone. ^eAggregate = baseline cohort + longitudinal sub-cohort. This total sample pool includes **192 unique subjects**.

Table 3: Discriminatory Power Across p-Tau 217 Intermediate Cases (n = 110)

Marker	AUC [95% CI]
Aβ42/40 Ratio	0.758 [0.67 – 0.83]
p-Tau 217	0.745 [0.67 – 0.82]
GFAP	0.698 [0.61 – 0.78]
NfL	0.439 [0.35 – 0.54]
LucentAD Complete Risk Score	0.856 [0.79 – 0.91]

Table 3. These metrics represent performance **specifically within the p-Tau 217 intermediate zone**, where standalone p-Tau 217 demonstrates reduced diagnostic certainty. The AUC of the integrated score (0.856) highlights the synergistic effect of combining neuroinflammatory (GFAP) and proteomic (Aβ42/40) markers to resolve biological ambiguity.

Table 4: Diagnostic Adjudication of the p-Tau 217 Intermediate Cases (n=110)

Algorithmic Classification	n	Amyloid Positive (PET+)	Amyloid Negative (PET-)	Concordance with PET
High Risk	38	32	6	84.2%
Low Risk	37	4	33	89.2%
Intermediate (Remaining)	35	15	20	N/A (Transition State)
Total	110	51	59	86.7% (Overall Accuracy*)

Table 4. Performance of the multi-analyte algorithm in resolving samples that fell within the intermediate range (0.04–0.09 pg/mL) of the p-tau 217 assay. High Risk (>70) and Low Risk (<45) tiers were adjudicated based on Aβ42/40, GFAP, and NFL. Remaining Intermediates are considered in an active biological transition state where biomarkers suggest nascent pathology that may not yet cross the threshold of PET positivity.

Table 5: Forensic Taxonomy of Intermediate Zone Adjudication (Examples)

Clinical Phenotype	Patient Case #	p-Tau 217 Intermediates (0.04-0.09)	Critical Secondary Signal(s)	Lucent Risk Score (45-70)	PET Status	Mechanism of Algorithmic Adjudication
Amyloid Rescue	1346	0.083	Low Aβ42/40 ratio: 0.039	88	High Risk (PET+)	A definitive drop in the amyloid ratio overrides a borderline tau signal.
Rule-Out Guard	2036	0.048	Low GFAP: 155 pg/mL	28	Low Risk (PET-)	Stable astrocyte and amyloid markers correct equivocal tau elevation.
Inflammation Divergence	1074	0.053	High GFAP: 742 pg/mL	91	High Risk (PET+)	Significant neuroinflammation (GFAP) drives the score to High Risk despite borderline tau.
Transition State	680	0.060	Midrange	58	Low Risk (PET-)	All biomarkers remain in the middle range, above normal but not pathological.

Table 5: Representative cases within the p-Tau 217 intermediate zone to illustrate mechanism of the multi-analyte adjudication. Comparison of cases 2036 and 1074 illustrates how disparate neuroinflammatory signals (GFAP) can drive a divergent risk assignment for patients with similar p-Tau 217 concentrations. The "Transition State" (case 680) example illustrates the algorithm's ability to identify patients with nascent biological activity that has not reached the threshold of PET positivity.

Conclusions

- These results establish diagnostic parity in clinical performance (**AUC, accuracy, sensitivity, specificity, NPV, and PPV**) between algorithmic immunoassay (LucentAD Complete) and algorithmic IP-MS assay (PrecivityAD2). Lucent AD Complete exhibited PPV robustness (92%) at an amyloid prevalence of 40.9%.
- LucentAD Complete reduced the intermediate zone of p-Tau 217 approximately 3-fold (aggregate 37.4% to 11.9%) by leveraging the full four-axis ATN(I) biomarker panel of p-Tau 217, Aβ42/40, NFL, and GFAP for accurate classification.
- Providing individual biomarker results alongside the composite Risk Score offers a more granular foundation for comprehensive patient management than amyloid status alone, supporting premium reimbursement for multi-analyte algorithmic BBMs in clinical practice.