LP046

Analytical Validation and Initial Clinical Evaluation of a New Blood-Based Diagnostic Test for Alzheimer's Disease

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OBJECTIVE

- To report the analytical validation and initial clinical performance of a plasma P-tau217 immunoassay test on a Quanterix SP-X platform for identifying amyloid PET-positive patients.

CONCLUSIONS

- This P-tau217 immunoassay using the Quanterix SP-X platform
  - has undergone systematic analytical validation,
  - demonstrates high positive and negative agreement with amyloid PET and,
  - could prove to be a useful diagnostic test to identify the presence or absence of amyloid pathology.

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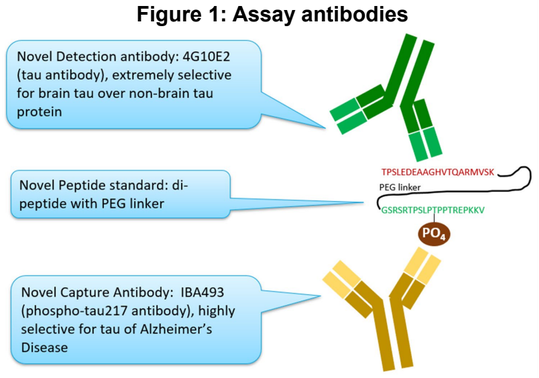
BACKGROUND

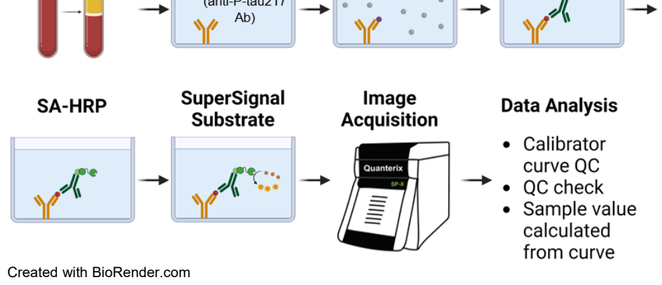
- Accessible, minimally invasive methods to detect AD pathology are lacking
  - Blood-based biomarkers have been gaining interest in the diagnostic work-up of neurodegenerative diseases, including AD
- Among the most interesting plasma biomarkers for AD is a post-translationally modified fragment of tau protein, P-tau217
  - Two previously characterized antibodies, 4G10E2 and IBA493, measure brain-specific tau-fragments [1]

Figure 1: Assay antibodies



METHODS

Figure 2: Workflow



Plasma P-tau217 chemiluminescent immunoassay

- Formal analytical validation consisting of three Quanterix manufactured P-tau217 kit lots, nine different operators, and six Quanterix SP-X imagers was performed in our CAP-accredited CLIA lab
- QC Acceptance Criteria
  1. Controls run twice on a plate
     - High, medium, and low controls are on every run in front and after patient samples on each plate
  2. 4/6 controls must pass and at least one control must pass at each level
     - ≤ 20% U/mL duplicate CV
     - ± 20% RE from target U/mL
  3. Ratio of controls must be within 0.85-1.15
     - Back and front controls are compared to check for plate bias
     - Average %RE of all controls passing duplicate %CV are used
- Standard Curve Criteria and Curve Fitting:
  1. 7 standards and a zero run on every plate
  2. Power (Log-Log) Regression is used to convert raw values to U/mL
     - 4/5-PL curve fit increased both intra- and inter-run variability
     - Power Regression maintained/reduced both intra- and inter-run variability
  3. 5/7 non-zero standards must pass
     - ≤20% raw duplicate CV
     - ±20 % RE from target U/mL
     - If multiple fail, the most egregious is removed first

ANALYTICAL VALIDATION

Precision

- 23 patient-derived precision samples, individual results ranged from 0.09 U/mL to 3.35 U/mL
- Assessed 80 precision runs across 25 days and multiple imagers with 3 total assay kits
- Total precision was ≤20% CV
  - Sample %CV ranges from:
    - 7.2 to 36.1% with QC applied
    - 7.3 to 14.3% with QC applied and outlier removal
  - Within laboratory precision was 11.5% CV (95% CI 10.98, 12.01) with QC applied
- No significant lot-to-lot differences were observed

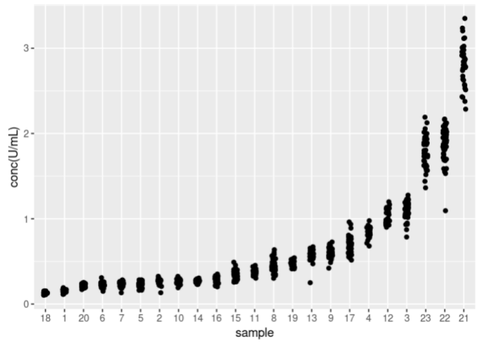
Table 1: Summary of within lab precision by reagent lot

Lot	Within Lab Precision (%CV)	95% CI
Lot 3	12.3	(11.25, 13.33)
Lot 4	9.2	(8.49, 9.80)
Lot 5	12.1	(11.07, 13.17)

Table 2: Summary of reagent lot contrasts

Mean Contrast	Ratio	90% CI
L3/L4	0.98	(0.97, 0.99)
L3/L5	0.97	(0.96, 0.99)
L4/L5	0.99	(0.98, 1.00)

Figure 3: Concentration per patient sample
Data from all passing plates without outlier removal



Instrument Comparison

- Multiple instruments demonstrated consistent results
  - A similar fail rate was observed for 5 of 6 instruments
  - One instrument was removed from the study due to a high fail rate

Parallelism and dilution linearity

- Parallelism and dilutional linearity support the use of a standard 1:2 dilution of plasma sample in diluent

Analytical Measurement Range

- 0.080 to 2.814 U/mL

Sample stability

- Samples are stable for up to 7 freeze/thaw cycles with room temperature and refrigerated stability for at least 72 hours

Analytical Specificity and Interference

- Irrelevant antibodies were assessed by using irrelevant capture and detection antibodies paired with original antibodies which resulted in errors and did not produce data
- There was no endogenous interference from tau441 (non-phosphorylated tau) (up to 20 ng/mL tested) or lipemia
- Hemolysis greater than 2+ was found not acceptable

Sensitivity

- Analytical sensitivity was established as
  - LoB of 0.041 U/mL
    - LoB was established using sample diluent buffer. A total of 178 blank reportable results were generated across 3 instruments, 3 reagent kit lots, 7 analysts and up to 3 unique days.
  - LoD of 0.070 U/mL
    - LoD experiments were performed by measuring 6 K2EDTA plasma samples with P-tau217 concentrations <0.2 U/mL and were tested in 10 replicates per run, 2 runs per day for 3 days to reach a total of 60 measurements per sample.
  - LLoQ 0.080 U/mL
    - LLOQ was defined through an acceptable precision approach according to CLSI guidance EP17A2E. Four separate low level samples were run 5 times per plate, across 15 plates (5 plates per lot), for a total of 75 replicate measurements per sample.

Figure 4: Aqueous Blank Concentrations Reported per Instrument
(colored by analyst)

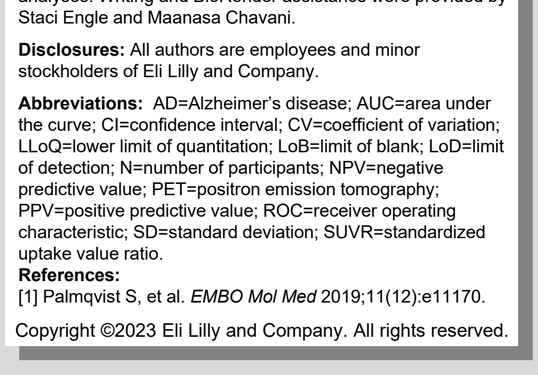


Table 3: LoB and LoD

Lot	LoB	LoD
Lot 3	0.025	0.059
Lot 4	0.035	0.065
Lot 5	0.041	0.062

Table 4: LLoQ
All lots combined

Donor ID	Mean (U/mL)	SD	CV	N
1	0.076	0.014	18.4%	75
2	0.051	0.013	24.5%	75
3	0.089	0.017	18.7%	75
4	0.076	0.016	20.4%	75

INITIAL CLINICAL EVALUATION

Predicting amyloid PET positivity

- Concentration of P-tau217 in plasma samples from a subset of individuals screened in TRAILBLAZER-ALZ 2 were compared to florbetapir SUVR
- The AUC of the plasma samples assayed was 91.6% (95% CI 0.90, 0.94) for predicting PET positivity
- Plasma P-tau217 concentrations used for ROC analysis from patients with known PET status follow closely overlapping, log-normal distributions.
- Using two post-hoc chosen thresholds, the PPV was 94%, the NPV was 86%, with 18% of samples being between the upper and lower thresholds

Table 5: Demographics

Demographic	N=1124
Age, mean (SD)	72.87 (6.47)
Female, n (%)	602 (53.7)
MMSE, mean (SD)	24.66 (2.52)
APOE ε4 Carrier, n (%)	573 (51.3)
Amyloid Positive (≥1.1 SUVR), n (%)	702 (64.3)

Figure 5: ROC Curve

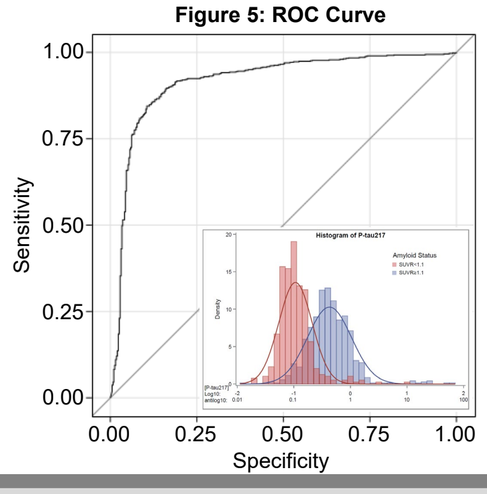
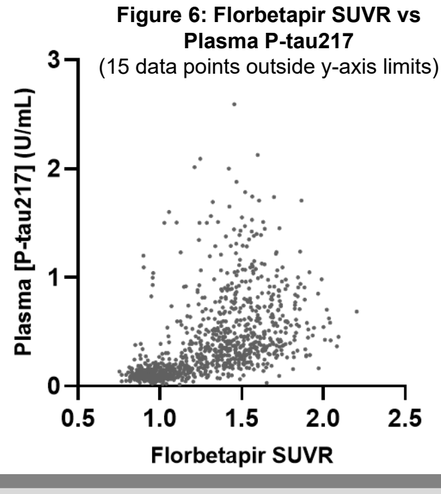


Figure 6: Florbetapir SUVR vs Plasma P-tau217
(15 data points outside y-axis limits)



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Abbreviations: AD=Alzheimer's disease; AUC=area under the curve; CI=confidence interval; CV=coefficient of variation; LLoQ=lower limit of quantitation; LoB=limit of blank; LoD=limit of detection; N=number of participants; NPV=negative predictive value; PET=positron emission tomography; PPV=positive predictive value; ROC=receiver operating characteristic; SD=standard deviation; SUVR=standardized uptake value ratio.

References:

[1] Palmqvist S, et al. *EMBO Mol Med* 2019;11(12):e11170.