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

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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
  - Plasma P-tau217 concentrations used for ROC

■ No significant lot-to-lot differences were observed
  - 23 patient-derived precision samples, individual results ranged from 0.09 U/mL to 3.35 U/mL
  - Total precision was ≤20% CV
  - Within laboratory precision was 11.5% CV (95% CI 10.98, 12.01) with QC applied

■ No significant lot-to-lot differences were observed
  - Two previously characterized antibodies, 4G8162 and IBA493, measure brain-specific tau-fragments [1]

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 images with 3 total assay kits

■ Total precision was 220% CV
  - Sample %CV ranges from:
    - 7.3 to 13.4% with QC applied
    - 7.3 to 13.4% 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

<table>
<thead>
<tr>
<th>Lot</th>
<th>Within Lab Precision (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 3</td>
<td>12.3</td>
<td>(11.25, 13.33)</td>
</tr>
<tr>
<td>Lot 4</td>
<td>9.2</td>
<td>(8.49, 9.80)</td>
</tr>
<tr>
<td>Lot 5</td>
<td>12.1</td>
<td>(11.07, 13.17)</td>
</tr>
</tbody>
</table>

Table 2: Summary of reagent lot contrasts

<table>
<thead>
<tr>
<th>Mean Contrast</th>
<th>Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3/L4</td>
<td>0.98</td>
<td>(0.97, 0.99)</td>
</tr>
<tr>
<td>L3/L5</td>
<td>0.97</td>
<td>(0.96, 0.99)</td>
</tr>
<tr>
<td>L4/L5</td>
<td>0.99</td>
<td>(0.98, 1.00)</td>
</tr>
</tbody>
</table>

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

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

Analytical Measurement Range

■ 0.010 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

Table 5: Demographics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>N=1124</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>72.87 (6.47)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>602 (53.7)</td>
</tr>
<tr>
<td>MMSE, mean (SD)</td>
<td>24.66 (2.52)</td>
</tr>
<tr>
<td>ApOe e4 Carrier, n (%)</td>
<td>573 (61.3)</td>
</tr>
<tr>
<td>Amyloid Positive (21.1 SUVR), n (%)</td>
<td>702 (64.3)</td>
</tr>
</tbody>
</table>

CONCLUSIONS

■ 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.

■ 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.

 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

■ 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
    - 4/5 controls must pass and at least one control must pass at each level
    - ≤20% U/mL, duplicate CV
    - ≤20% RE from target U/mL
    - Back and front controls are compared to check for plate bias
    - Average %RE of all controls passing duplicate %CV are used
  - 2. 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

■ Instrument Comparison
  - Multiple instruments demonstrated consistent results
    - Sensitivity: 94%, specificity: 86%
    - Analysis was performed on 10 samples across 3 instruments, 3 reagent kit lots, 7 analysts and up to 3 unique days
  - Typical variation was observed.

■ Standard Curve Criteria and Curve Fitting:
  - 7 standards and a zero run on every plate
  - 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

■ 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
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■ Power Regression maintained/reduced both intra- and inter-run variability

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■ Analytical sensitivity was established as
  - LoB of 0.041 U/mL
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■ LoD of 0.070 U/mL
  - LoD experiments were performed by measuring 6K2EDA 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.

■ LLq 0.080 U/mL
  - LLq 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 2: Workflow
  - Figure 3: Concentration per patient sample
  - Figure 4: Aqueous Blank
  - Figure 5: ROC Curve
  - Figure 6: Florbetapir SUVR vs Plasma P-tau217

ACKNOWLEDGMENTS

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Abbreviations: AD = Alzheimer’s disease; AUC = area under the curve; CI = confidence interval; CV = coefficient of variation; LLq = lower limit of quantitation; LoD = 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:

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