SpearBio

Distributed By
biotechne®

APPLICATION NOTE

Unlocking the potential of pTau 231 in Alzheimer's disease research with the enhanced sensitivity and precision of the SPEAR UltraDetect™ pTau 231 Assay

Abstract

Phosphorylated tau at threonine 231 (pTau 231) is emerging as a promising blood-based biomarker for incipient Alzheimer's disease (AD) pathology—yet the sensitivity of existing assays like Simoa® is still inadequate to reliably quantify blood pTau 231 in healthy individuals, with reported 78% and 40% detectability and quantifiability, respectively.

In this application note, we compare the analytical performance of SPEAR UltraDetect™ pTau 231 Assay with Simoa pTau 213 Advantage PLUS Assay in measuring plasma pTau 231. The SPEAR assay demonstrates higher sensitivity and precision, achieving 100% detection and 100% quantification in healthy individuals and greater precision (Average CV 4.2% vs. 13.8% for Simoa). With enhanced sensitivity and precision, SPEAR UltraDetect enables robust quantification of baseline and subthreshold changes in pTau 231 levels, unlocking the potential of pTau 231 as a plasma biomarker for early AD-detection and longitudinal tracking of disease progression.

Introduction

Early and accurate detection of AD pathology is critical for advancing therapeutic development and improving patient outcomes. pTau 231 has been shown to strongly associate with the earliest cerebral amyloid-beta (Aβ) changes in AD, with plasma pTau 231 becoming abnormal at preclinical stages with lowest Aβ burden, compared to other key biomarkers such as pTau217 and pTau181, before the threshold of Aβ-PET positivity is reached.[1,2] These data present pTau 231 as a strong candidate biomarker for early AD detection and for tracking longitudinal Aβ changes in clinical studies.

However, the potential for pTau 231 to function as a minimally invasive biomarker has been limited. This is because existing pTau 231 immunoassays lack adequate sensitivity and precision to reliably measure the baseline of pTau 231 in blood from healthy and preclinical AD individuals, making it challenging to establish pTau 231 as a blood-based AD biomarker.

Successive Proximity Extension Amplification Reaction (SPEAR) is a homogeneous, ultra-sensitive immunoassay technology designed to overcome the sensitivity limitations and complexity of conventional immunoassays. Utilizing a unique two-factor authentication mechanism, SPEAR ensures that amplifiable signal is only generated through sustained co-localization of probes on target proteins. It precisely quantifies protein biomarkers at attomolar concentrations from as

Introduction, continued

little as as 1 µl of diluted sample, greatly surpassing the most sensitive heterogenous platform on the market. The homogeneous nature of SPEAR eliminates non-specific binding associated with solid surface capture which gives it superior specificity over heterogeneous immunoassay platforms. SPEAR requires no error-prone wash steps in the workflow, ensuring its extremely high precision.

Powered by this core technology, SPEAR UltraDetect™ assays offer unparalleled sensitivity in measuring low-abundance biomarkers with exceptional specificity and precision. SPEAR UltraDetect utilizes standard qPCR instruments for readout, enabling highly consistent results across different qPCR platforms and formats. It is easy to implement into existing laboratory settings with unprecedented scalability.

Here we compare the performance of the SPEAR UltraDetect pTau 231 Assay with the Simoa pTau 231 Advantage PLUS assay, demonstrating higher sensitivity and precision in plasma samples.

EDTA plasma from 25 apparently healthy donors (A-) and 15 diagnosed AD individuals (A+) were obtained from commercial sources and tested using SPEAR UltraDetect™ pTau 231 (item SPR90009, Spear Bio) and Simoa® pTau 231 Advantage PLUS (item 104512, Quanterix).

SPEAR UltraDetect™ Method

Samples were processed using the Formulatrix® F.A.S.T™ liquid handler and QInstruments® BioShake® iQ, following SpearBio's instructions. Briefly, a 1 µL probe mix was combined with 1 µL of sample or calibrator and incubated for 1 hour. 6 µL reaction mix was added and incubated for 20 minutes, followed by addition of 12 µL of qPCR mix for final analysis. qPCR results were converted to protein concentrations using SPEARview analysis software. (Figure 1)

Materials and Methods

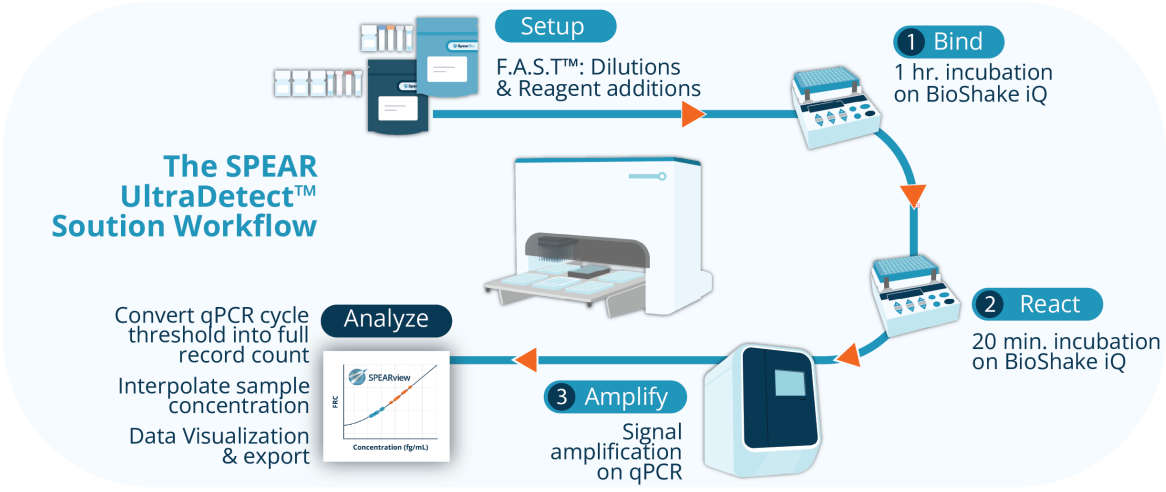

Figure 1. The full SPEAR UltraDetect Assay workflow.

Materials and Methods, continued

Quanterix Simoa® Method

Samples were run on the Simoa HD-X Analyzer® per the manufacturer's protocol. Briefly, 100 µL of diluted sample or calibrator was mixed with 25 µL bead reagent, followed by washing (3x), addition of 100 µL detection reagent, washing (3x), addition of 100 µL SBG reagent, washing (4x), and addition of 50 µL RGP. Protein measurements were generated using the Quanterix software.

Results

The SPEAR UltraDetect pTau 231 Assay delivers 100% detectability and 97% quantifiability in plasma samples of healthy individuals based on the assay verification study (Table 1)[3], representing a markedly improved sensitivity compared to the reported 78% and 40%, respectively, of the Simoa pTau 231 Advantage PLUS Assay[4].

Table 1: SPEAR UltraDetect™ pTau 231 Assay specifications in EDTA plasma samples.

Specification	Values
MRD	4-fold
Diluted sample volume	1 µL
Raw sample volume to machine*	10 µL
Analytical LLoD (range)	0.039 pg/mL (0.022-0.106)
Functional LLoD	0.157 pg/mL
Functional LLoQ	0.330 pg/mL
Functional Assay Range	0.330-2640 pg/mL
Average Intra-plate CV	2.8%
Average Inter-plate CV	6.5%
Spike Recovery (Range)	103% (79-111%)
Dilution Linearity (Range)	89% (86-95%)
% above LLoD/Detectability (Number of donors)	100% (n=34)
% above LLoQ/Quantifiability (Number of donors)	97% (n=34)

*Can be used for multiple replicates and measurements.

When measuring 40 plasma samples from a same cohort on the two platforms, all 25 non-AD samples tested were measured at concentrations above LLoQ by SPEAR, while 7 (28%) were below LLoQ of the Simoa assay (Figure 2). Additionally, the SPEAR measurements exhibited higher precision with an average CV of 4.2% across all 40 samples, whereas Simoa-measured samples returned an average CV of 13.8% (Figure 2).

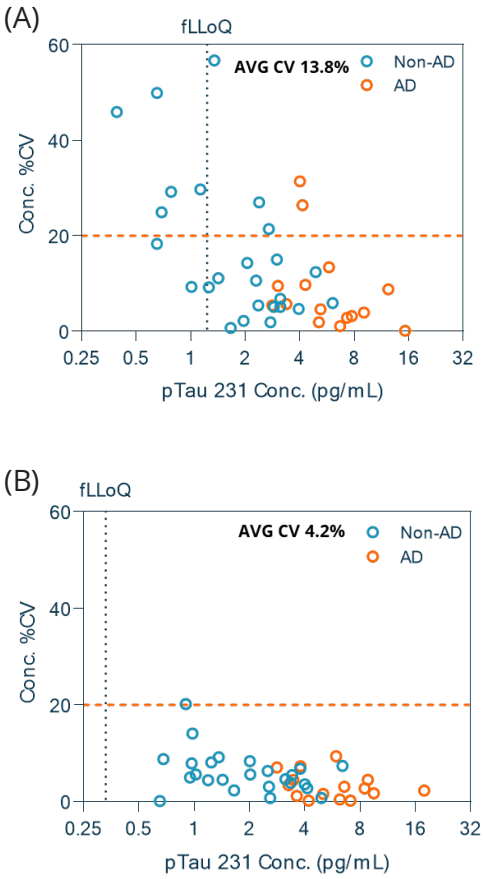

Figure 2: Sample precision profile of **(A)** Simoa® pTau 231 Advantage PLUS and **(B)** SPEAR UltraDetect pTau 231, respectively, in plasma samples from AD (n=15) and non-AD (n=40) individuals. Functional LLoQ (fLLoQ) and 20% CV reference lines are indicated by dashed lines.

The mean of SPEAR-quantified pTau 231 revealed a 3.1-fold difference between AD and healthy control (Figure 3).

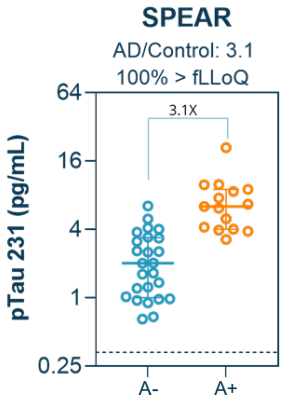

Figure 3: pTau 231 concentrations measured using the SPEAR UltraDetect pTau 231 Assay in A- (n=25) and A+ (n=15), and the difference between the mean of concentrations of the two groups. The fLLoQ is indicated by the dashed line. Bars indicate median with interquartile range.

Discussion

In this comparison study, SPEAR UltraDetect™ pTau 231 demonstrated superior sensitivity and precision while using 100-fold less sample volume over Simoa® pTau 231 Advantage PLUS, achieving 100% quantification with 1 µL of diluted sample in all 40 plasma samples tested. Additionally, a 3.1-fold difference in mean pTau 231 concentrations between AD and control groups highlights the assay's strong potential to distinguish amyloid-positive from amyloid-negative individuals.

The combined gain in sensitivity and precision is critical for detecting subtle, early shifts in plasma pTau 231 associated with Aβ accumulation—enabling earlier disease detection and more reliable tracking of disease progression well before clinical symptoms appear. In clinical research settings, SPEAR UltraDetect pTau 231 offers a powerful tool for enriching study subjects by identifying individuals in the earliest stages of disease and for evaluating amyloid burden in response to disease-modifying therapies.

References

1. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. https://link.springer.com/article/10.1007/s00401-021-02275-6

2. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. https://pubmed.ncbi.nlm.nih.gov/35953717/

3. SPEAR UltraDetect™ pTau 231 Datasheet (SPR90009) (https://24277728.fs1.hubspotusercontent-na1.net/hubfs/24277728/Datasheet_SPEAR%20UltraDetect%20pTau%20231%20v1.pdf)

4. https://www.quanterix.com/wp-content/uploads/2024/03/p-Tau-231-Advantage-PLUS-Data-Sheet.pdf

SpearBio

100 Tradecenter Dr. Suite 510
Woburn, MA 01801

spear.bio

SPR DOC80053

Distributed by:
bio-techne®

614 McKinley Place NE
Minneapolis, MN 55413

bio-techne.com
1-800-343-7475