SPEAR UltraDetect™ pTau 217: A High-Accuracy, Scalable Plasma Biomarker for PET Confirmed Cognitively Impaired Patients Using a Single Cutoff

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Introduction

Misdiagnosis of Alzheimer's disease in both memory clinics and primary care often delays confirmatory testing and shortens critical treatment windows for anti-amyloid therapies. Plasma pTau 217 assays offer a minimally invasive, scalable option to detect amyloid pathology, but most fall short of achieving >90% sensitivity and specificity with a single cutoff. Instead, they use tiered "double-cutoff" methods that discard an intermediate "gray zone" (over 20% of cases), and stricter thresholds further expand this zone—leaving many patients in diagnostic limbo.

We present the SPEAR UltraDetect™ pTau 217 immunoassay, which employs a unique two-factor authentication mechanism and a homogenous assay format allowing free analyte-binder interaction for maximum specificity. This semi-automated test measures analyte in just 1µL of diluted plasma on a wash-free workflow with qPCR readout. In a Global Alzheimer's Platform (GAP) cohort (MCI, n=67; mild dementia, n=34; amyloid PET–confirmed negative, n=44; positive, n=57), SPEAR achieved >90% clinical accuracy for identifying amyloid pathology using a single cutoff.

Methods

SPEAR Two-Factor Authentication Platform

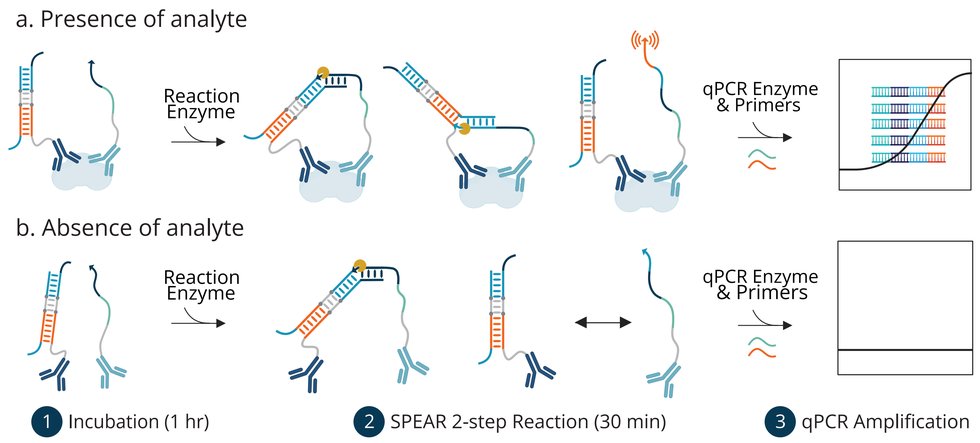

Fig. 1. The Successive Proximity Extension Amplification Reaction (SPEAR) uses two antibody probes conjugated to DNA devices. One contains two toehold regions (dark blue and cyan) while the other contains a sequence complementary to a toehold of the double stranded device (dark blue). Top: When both antibodies bind pTau 217, their proximity enables hybridization of one toehold region and DNA polymerase extension adds a sequence complementary to the second toehold. As the analyte binding maintains proximity, a second toehold hybridization and extension occurs to create an amplifiable DNA sequence. Bottom: Transient hybridization may occur for the first extension, but without the target binding them in place, the antibodies separate and the second hybridization does not occur, leaving an unamplifiable DNA sequence.

SPEAR UltraDetect pTau 217 Workflow and Performance

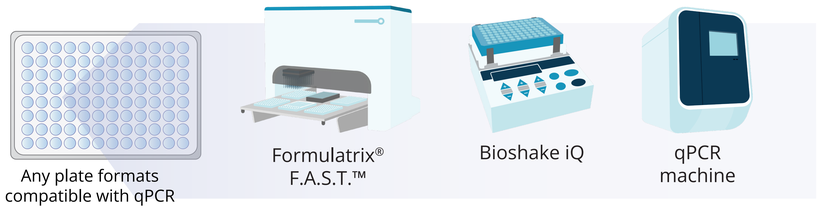

Fig. 2. The SPEAR assay workflow is lab-deployable, requiring standard lab equipment, and the wash-free workflow is easily adapted to automation.

Table 1. SPEAR UltraDetect pTau 217 Specifications

MRD	4x (EDTA Plasma)	
Diluted Sample Vol.	1 µL	
Sensitivity	**Analytical**	**Functional**
LLoD (fg/mL)	4.9	19.7
LLoQ (fg/mL)	20.0	80.0
Assay Range (fg/mL)	20.0-160000	80.0-640000
Precision (n=6)	**Inter-assay**	**Intra-assay**
Mean (Range)	4.4% (2.9-6.7%)	3.3% (1.5-10.0%)
Matrix Effects (n=3)	**Spike-in Recovery**	**Dilution Linearity**
Mean (Range)	106% (92-126%)	87% (77-95%)
Interference	**Similar Protein**	**Interferant**
	Tau,pT181,pT231	Hb, TG, CB,UB
	Not Significant	Not Significant

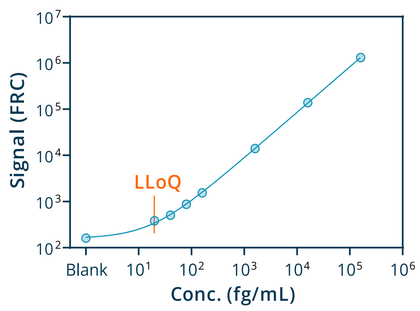

Fig. 3. Representative calibration curve fitted with 4PL $1/y^2$ weighting. Analytical LLoQ indicated in orange.

Cohort Data

Table 2. GAP Cohort Demographics

	Amyloid PET +			Amyloid PET -		
Cognitive Status	MCI	AD	Total	MCI	AD	Total
Population (n)	33	24	57	34	10	44
Age						
Mean (SD)	71.8 (6.1)	75.1 (5.3)	73.2 (5.9)	72.9 (7.3)	73.5 (5.6)	73.0 (6.9)
Sex, n (%)						
Female	16 (48%)	12 (50%)	28 (49%)	17 (50%)	5 (50%)	22 (50%)
Race, n (%)						
White	30 (91%)	22 (92%)	52 (91%)	28 (82%)	7 (70%)	35 (80%)
Ethnicity, n (%)						
Hispanic/Latino	5 (15%)	5 (21%)	10 (18%)	4 (12%)	2 (20%)	6 (14%)
MMSE						
Mean (SD)	26.5 (1.9)	22.4 (2.5)	24.8 (3.0)	27.5 (1.7)	24.8 (1.2)	26.9 (2.4)
APOE, n (%)						
E3/E4	22 (67%)	7 (29%)	29 (51%)	9 (26%)	1 (10%)	10 (23%)
E4/E4	3 (9%)	4 (17%)	7 (12%)	0 (0%)	1 (10%)	1 (2%)
Centiloid						
Mean (SD)	82.5 (39.9)	96.8 (37.5)	88.4 (39.2)	-5.0 (16.9)	5.4 (26.1)	-2.9 (19.3)
Missing Data	2 (6%)	2 (8%)	4 (7%)	0 (0%)	1 (10%)	1 (2%)

Results

SPEAR Assay Shows Superior Sensitivity and Specificity

A greater fold separation between amyloid-PET negative and positive populations (4.86 for SPEAR, 2.70 for Eli Lilly MSD) leads to a higher accuracy (92% vs. 88%) when establishing a single cutoff.

For applications employing a double-cutoff approach with threshold settings at 95% sensitivity and specificity, the SPEAR assay demonstrates an increase in statistical performance while still keeping the intermediate zone to below 20% vs. 34% in the MSD assay with Lilly antibody, at a lower performance.

The high analytical sensitivity of SPEAR also enables 100% quantifiability across the cohort.

Table 3. SPEAR and Eli Lilly MSD assay statistical performance for double cutoff and single cutoff methods, and percentage of samples above the functional LLoQ.

	SPEAR		Eli Lilly MSD	
Cutoff Scheme	Double	Single	Double	Single
Sensitivity	94%	91%	91%	87%
Specificity	94%	93%	92%	88%
PPV	96%	95%	94%	92%
NPV	92%	89%	89%	81%
Accuracy	94%	92%	91%	88%
Indeterminate Range	12%	-	34%	-
Quantifiability	100%		89%	

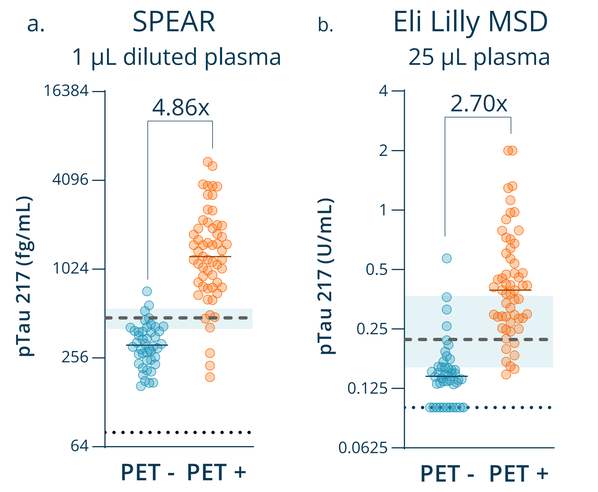

Fig. 4. SPEAR assay (a.) and Eli Lilly MSD assay (b.) results showing optimal single cutoff (gray dashed) and double cutoff (blue shaded) methods. The fold change between the mean value between PET+ and PET- are indicated above the bracket. The functional LLoQ is indicated by a dotted line.

ROC Analysis and Correlation with Aβ-PET Shows Excellent Performance

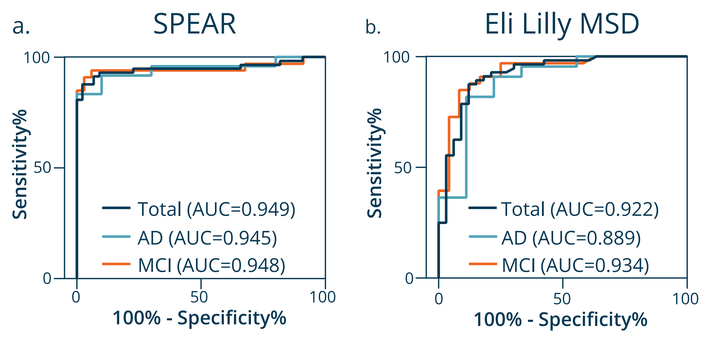

Fig. 5. SPEAR assay (a.) and Eli Lilly MSD assay (b.) ROC analysis for the single cutoff method, separated by AD group, MCI group, and total cohort.

The calculated AUC for total cohort, MCI, and AD subdivisions is shown to be higher for the SPEAR assay as compared to the Eli Lilly MSD assay.

The SPEAR assay demonstrated a strong correlation with amyloid burden, with a Spearman's r of 0.802.

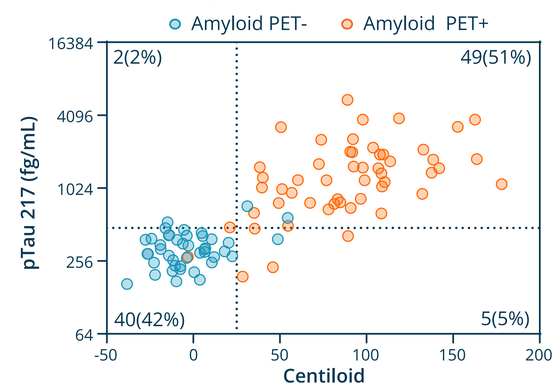

Fig. 6. SPEAR assay plot of samples with both pTau 217 measurements and centiloid measurements. Vertical line indicates centiloid equal to 25, and horizontal line indicates single cutoff point for the assay. Quadrants labeled with number of values (percentage of total).

Interplate %CV Shows Consistency Across Both Groups

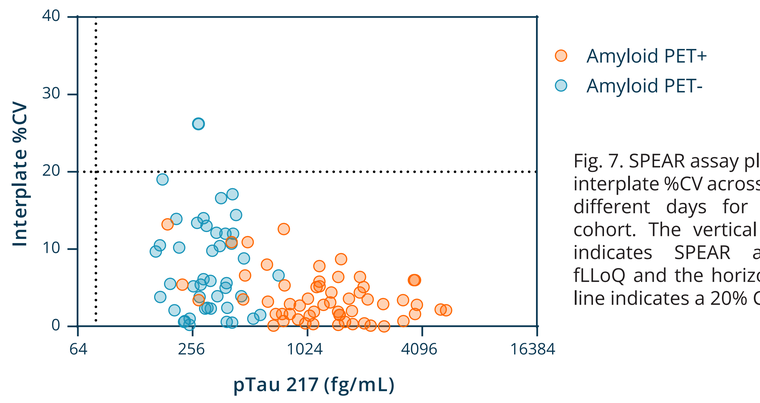

Fig. 7. SPEAR assay plot of interplate %CV across two different days for GAP cohort. The vertical line indicates SPEAR assay fLLoQ and the horizontal line indicates a 20% CV.

Table 4. SPEAR assay interplate sample %CV across two runs.

	PET-	PET+	Total
Average %CV	7.5%	3.7%	5.4%
% Sample CV <20%	98%	100%	99%
% Sample CV <15%	91%	100%	96%

Using a semi-automated workflow and separate sample aliquots measuring with 1 µL of diluted sample volume, the SPEAR assay achieves an interplate precision across two runs across two different days of 5.4%.

Across the GAP cohort, 96% of samples have an interplate CV below 15%.

Conclusions

The SPEAR assay demonstrated a 4.9-fold increase in pTau-217 levels in PET-positive cases, nearly doubling the 2.5-fold seen with the Eli Lilly MSD assay. The assay quantified all samples across the cohort and exhibited robust inter-day precision at 5.4% average CV. Using a single cutoff, SPEAR achieved 91.2% sensitivity, 93.2% specificity, 94.6% PPV, and 89.1% NPV, with 92.1% overall accuracy. When optimizing for ≥95% specificity and sensitivity, the number of points in the indeterminate range was reduced from 34% for the Eli Lilly MSD assay to 12% for the SPEAR assay.

The SPEAR UltraDetect pTau 217 assay offers improved clinical differentiation of amyloid PET status with >90% accuracy using a single cutoff. Its minimal sample requirement of 1 µL diluted plasma, wash-free semi-automated workflow, and qPCR compatibility make it a scalable, cost-effective test for determining amyloid pathology of Alzheimer's Disease.

Acknowledgement

K2EDTA plasma samples and associated data were part of the Bio-Hermes Study provided by Global Alzheimer's Platform Foundation, Washington, DC, USA.

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