Rapid Analysis and Validation of Antibody Pairs in a Few Days' Journey: Developing a pTau-217 Assay Using Fiber Optic Particle Plasmon Resonance (FOPPR) Technology

Ting-Chau A. Chang, PhD[1], Feng-Yi Ke, PhD[1], Po-Jen Chien, PhD[1], Tsun-Chih Chung[1], Lauren Chaby, PhD[2], Andreas Jeromin, PhD[2]

1.Instant Nanobiosensors Co.,Ltd., National Biotechnology Research Park, Taipei City, Taiwan

2. ALZpath, Inc., Carlsbad, California, USA

Keywords: Fiber-Optic Partical Plasmon Resonance, FOPPR, P-Tau217, PTMs, FONLISA

Theme: Biomarkers-Non-Neuroimaging

Email: INB@instantnano.com

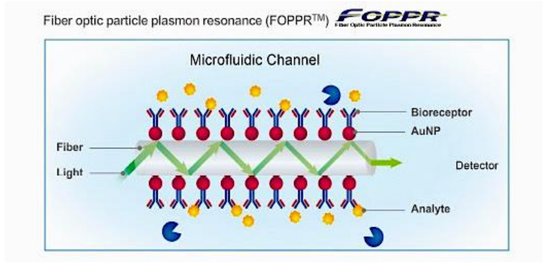
LinkedIn

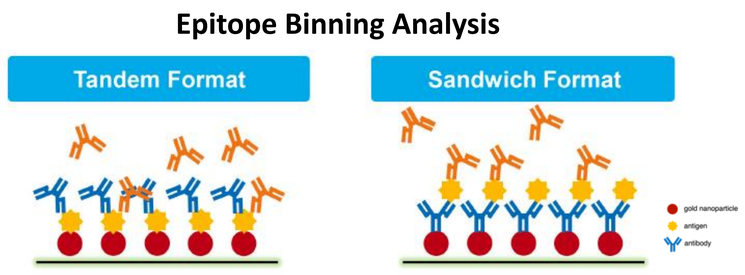
Background:

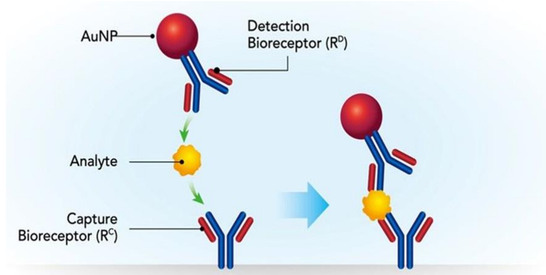
The development of a pTau-217 assay leveraging Fiber Optic Particle Plasmon Resonance (FOPPR) technology is designed to detect the abnormally phosphorylated tau protein variant, pTau-217, which is significant in neurodegenerative diseases like Alzheimer's. The goal is to establish pTau-217 as a reliable biomarker for point-of-care (POC) usage in both acute clinical settings and patient monitoring. This involves a meticulous process of identifying the most suitable pTau-217 antibody pairs through in-depth affinity and epitope binning analyses. FOPPR technology facilitates the effective analysis of antibodies that have undergone post-translational modifications (PTMs), aiding in the selection of the optimal antibody pair. Following this, the pTau-217 levels in EDTA plasma samples are quantified with enhanced precision and sensitivity utilizing the Fiber Optic Nanogold-linked Immunosorbent Assay (FONLISA) method.

Methods:

The FOPPR technology incorporates a carefully designed self-assembled monolayer (SAM) to mitigate retention effects and minimize rebinding, ensuring the accuracy of the measured affinity constants. Additionally, the system employs an auto-flowing microfluidic chip that mimics the velocity of blood through veins, simulating the physiological environment of the body. This approach allows for the evaluation of binding affinities under conditions that closely resemble real-world biological interactions. For the pTau-217 assay, nine antibodies were initially tested for their affinity for Tau protein, and two were excluded due to low affinity. The remaining antibodies were ranked by their affinity constants through epitope binning to identify their binding sites on Tau N-terminal or pTau-217. In FOPPR technology, the optical fiber set in a microfluidic chip detects nanoplasmonic absorption changes caused by the formation of the immunocomplex, using the INB D200 device. The assay's sensitivity (LLOD) was ascertained through blank measurements, with its linear range established using a pTau-217 calibrator.







Results:

The affinity constants for the antibodies ranged from a high of 0.4 nM to a low of 655 nM, with one antibody not yielding results. Epitope binning identified that antibodies A2, I2 (polyclonal), and ALZpath detection antibody, as well as B1 and A2-1, recognized the same epitope of the pTau-217 calibrator while antibody I1 (polyclonal) bound to a different epitope. The monoclonal ALZpath detection antibody and ALZpath capture antibody demonstrated the strongest affinity for the pTau-217 calibrator. For the assay, the ALZpath detection antibody was immobilized on the optical fiber core, and the ALZpath capture antibody was attached to gold nanoparticles. This setup provided a linear detection range from 0.0024 pg/mL to 10 pg/mL for pTau-217, with the lower limit of detection (LLOD) being less than 2.4 fg/mL. Recovery and CV in a duplicated spike test of the pTau-217 calibrator showed 96.8% recovery with a CV of 0.6% at low concentration (0.096 pg/mL), and a recovery of 101.8% with a CV of 2.7% at a higher concentration.

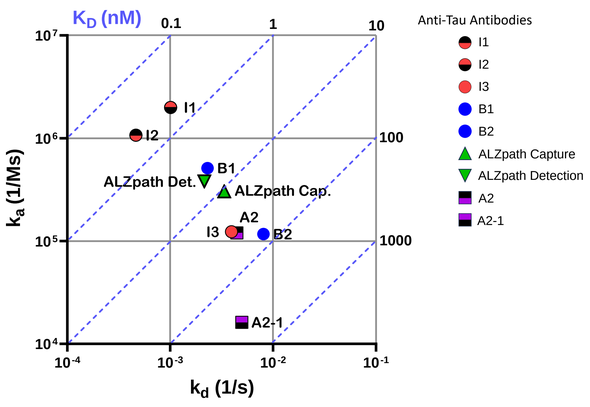


Figure 1. The affinity distribution of nine antibodies. The antibodies ALZ capture antibody, A2, and I3 recognize pTau-217, while the rest recognize the N-terminal of Tau.

Table 1. Quantitative measurement of antibody affinity

Antibody	I1 (poly)	I2 (poly)	I3	B1	B2	ALZpath capture	ALZpath detection	A2	A2-1
ka (1/Ms)	1.99×10^{6}	1.07×10^{6}	1.23×10^{5}	3.43×10^{3}	5.12×10^{5}	3.04×10^{5}	3.81×10^{5}	1.20×10^{5}	1.62×10^{4}
kd (1/s)	1.01×10^{-3}	4.65×10^{-4}	3.95×10^{-3}	2.25×10^{-3}	2.31×10^{-3}	3.35×10^{-3}	2.15×10^{-3}	4.45×10^{-3}	4.97×10^{-3}
KD (nM)	0.51	0.43	32.2	655	4.51	11.02	5.64	37.19	308

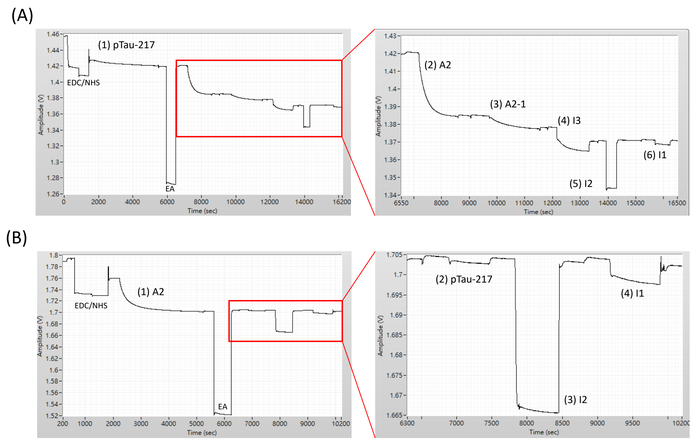


Figure 2. The Sensorgram of epitope binning. (A) The tandem format and (B) Sandwich format of epitope binning analysis. EDC/NHS to active carboxylic acid for immobilize protein on optic fiber. Ethylenediamine(EA) add to interactive with excess carboxylic acid.

Tau Antibody	Second Antibody: I1	I2	B1	B2	ALZ Det Ab	A2	A2-1
First Antibody: I1		non-blocking	non-blocking			non-blocking	
I2	non-blocking					blocking	
B1	non-blocking			non-blocking	blocking		partial blocking
B2			non-blocking		partial blocking		non-blocking
ALZ Det Ab			blocking	non-blocking			non-blocking
A2	non-blocking	blocking					non-blocking
A2-1			blocking	non-blocking	non-blocking	non-blocking	

Figure 3. The epitope binning map of these four antibodies. I3 and ALZpath capture antibody and did not undergo epitope binning due to material issues.

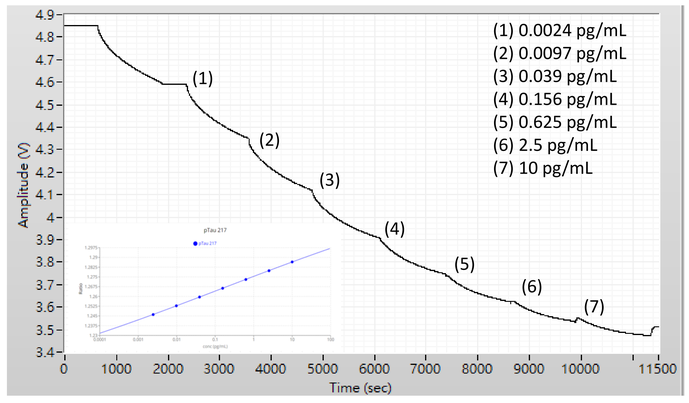


Figure 4. Real-time detection with ALZpath antibody pair for calibration curve upon injection of different pTau-217 calibrator concentrations ranging from 0.0024 pg/mL to 10 pg/mL (~4 orders)

Table 2. Spiked Test with ALZpath antibody pair on FOPPR technology

pTau 217 Spiked Test (n = 2)			
Prepared Conc. (pg/mL)	Average Conc. (pg/mL)	Recovery (%)	CV (%)
0.096	0.093	96.8%	0.6%
0.58	0.57	101.8%	2.7%

Conclusion:

The assay, based on FOPPR technology, effectively selected appropriate antibody pairs and demonstrated ultra-sensitive detection of pTau-217 in EDTA plasma. From initial antibody analysis to the validation of the antibody pair performance, preliminary results were typically obtained within a few days. The assay showed high specificity for pTau-217, with minimal cross-reactivity with other tau isoforms. Compared to existing methods, this assay offers a more sensitive and rapid detection of pTau-217, highlighting its potential for early diagnosis and monitoring of neurodegenerative diseases. The strong potential for POC applications is supported by continuous improvements aimed at enhancing technical stability and analytical sensitivity. Future work will focus on further validation of the assay in larger clinical cohorts and exploring its utility in monitoring disease progression and treatment response.