

# A QUANTITATIVE REAL-TIME IMMUNO-PCR TO MEASURE TAU PHOSPHORYLATED AT SERINE 199 IN CEREBROSPINAL FLUID

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## Introduction

Cerebrospinal fluid (CSF) level of abnormally hyperphosphorylated tau is believed to be a useful marker of Alzheimer neurofibrillary degeneration. However, tau in Alzheimer disease (AD) is abnormally hyperphosphorylated at several different specific sites and at different levels. To date, the most widely used assay is INNOTEST PhosphoTau (181P), which measures phosphorylation of tau at threonine 181 using 75 µL CSF/assay.

Here we describe an ultrasensitive quantitative immuno-PCR (qIPCR) assay allowing to measure the level of PhosphoTau (pTau) using only 2.5µL CSF/assay.

## Materials and Methods

### Patients

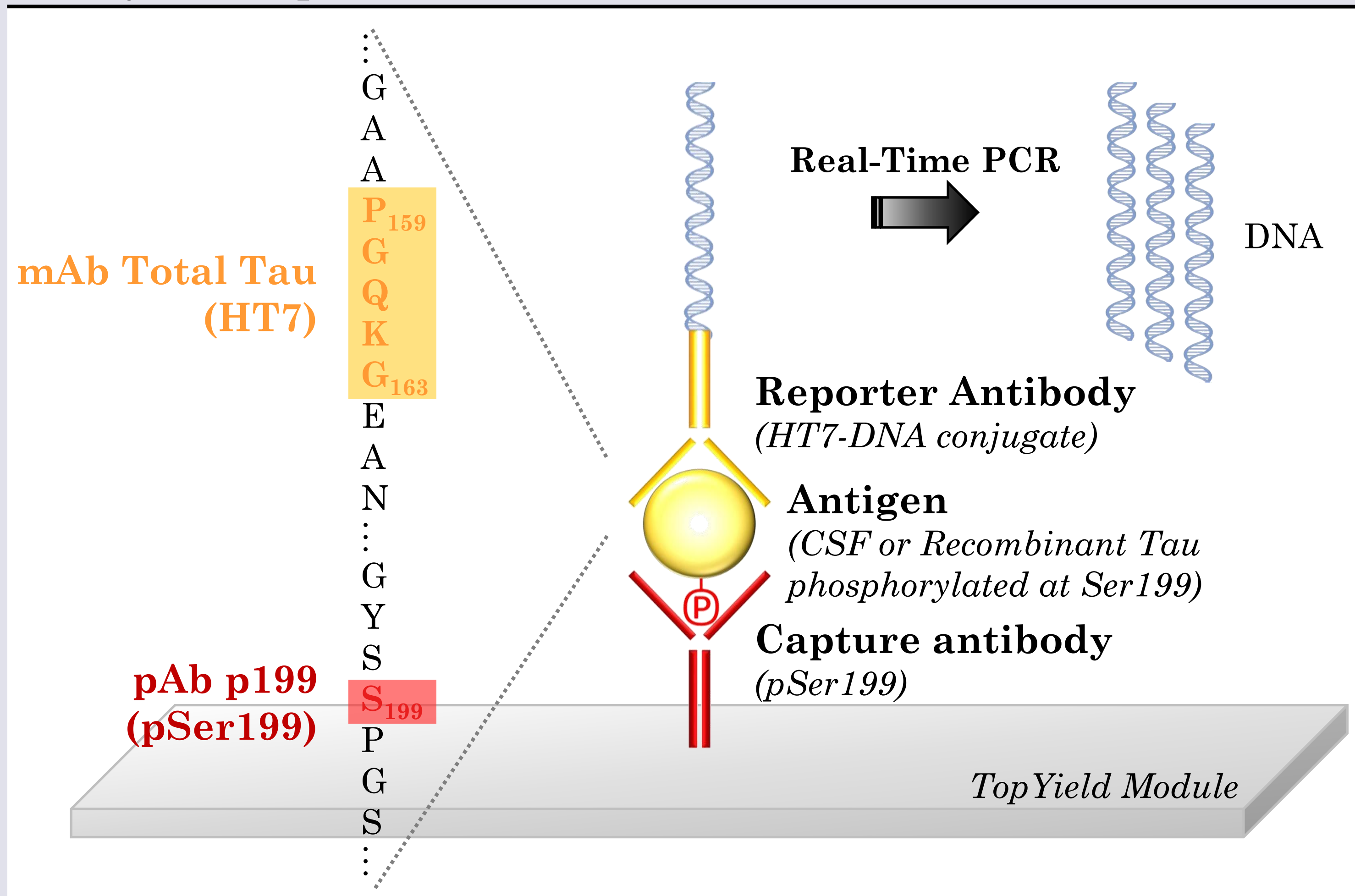
	Braak Stages		
	I-II	III-IV	V-VI
<b>n</b>	6	7	20
<b>Autopsy-based diagnostic<sup>(a)</sup> (Non-AD / AD)</b>	6/0 <sup>‡</sup>	2/5	0/20 <sup>‡</sup>
<b>CERAD<sup>(b)</sup> (A/B/C)</b>	2 <sup>†</sup> /1/3	0/2/5	0/2/18
<b>α-Synuclein (+/-)</b>	3/3	3/4	12/8
<b>Gender (Male/Female)</b>	4/2 <sup>‡</sup>	3/4	0/20 <sup>‡</sup>
<b>Duration (years)</b>	6.8±2.3	6.7±5.5	7.9±4.5
<b>ApoE4 Carrier (+/-)</b>	4/2	4/3	16/4
<b>PMD (hours)</b>	6±4	4±2	7±5
<b>Brain Weight (g)</b>	1146±153	1097±156	1005±113

Data are given as mean ± SD or as number of subjects in the group. Differences among Braak Stages regarding duration, PMD and brain weight were assessed using the Kruskal-Wallis test. Difference among Braak Stages regarding autopsy-based diagnostic, CERAD criteria, α-synuclein and ApoE4 distribution were assessed using the  $\chi^2$  test.

(†)  $p < 0.05$ , and (‡)  $p < 0.001$ : corresponding Braak stage vs the other Braak stages. (a) The Non-AD group includes dementia with Lewy bodies and vascular dementia cases. The AD group includes AD, AD of Lewy body type and AD of vascular disease type cases; (b) Senile plaques were evaluated according to the CERAD criteria and were classified into three stages: A, B and C indicating sparse, moderate and frequent, respectively.

Abbreviations: CERAD, Consortium to Establish a Registry for Alzheimer's disease; PMD, Postmortem delay; +, positive; -, negative.

### Assay Principle



## Results

### Assay Optimization

Fig 1. Procedure

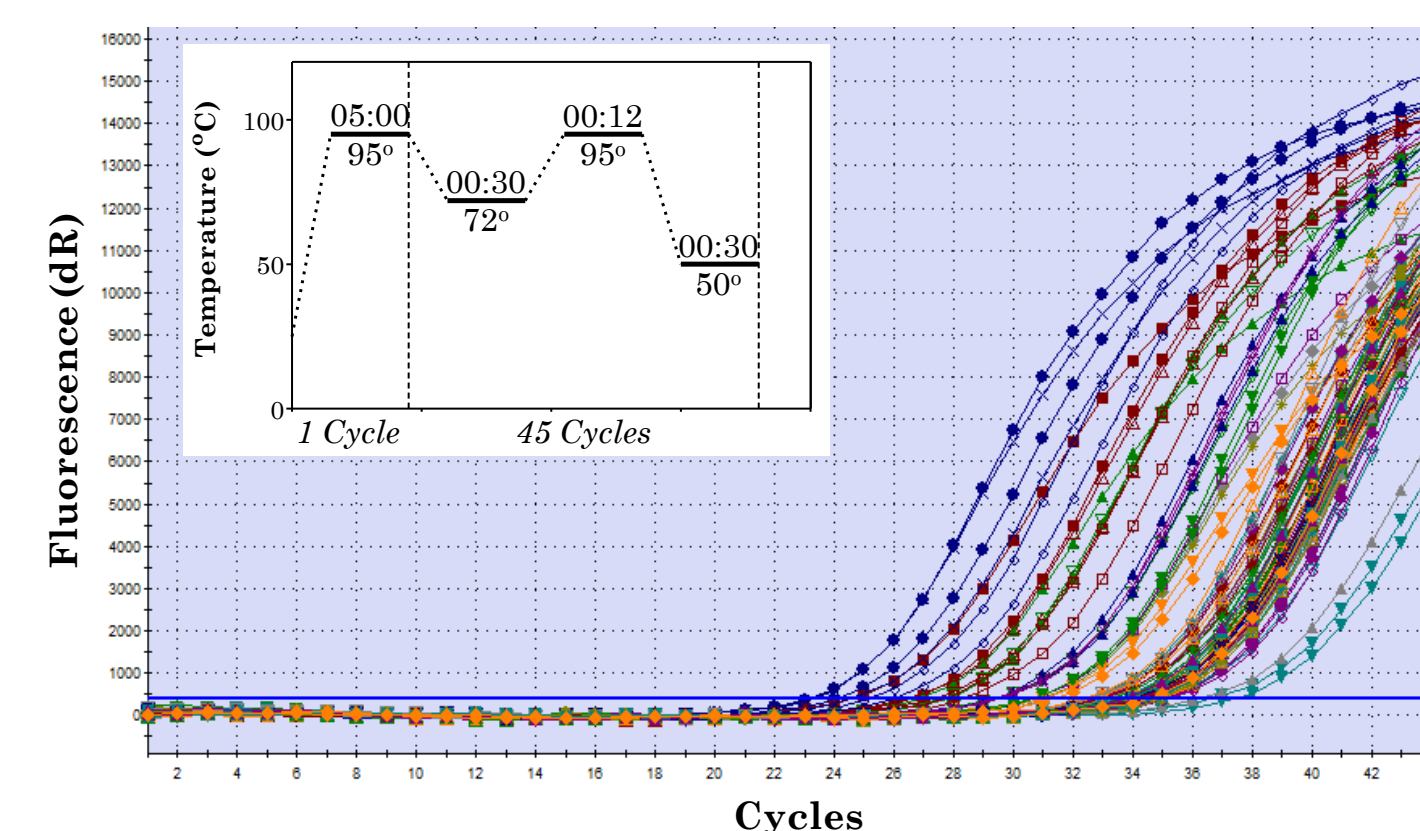
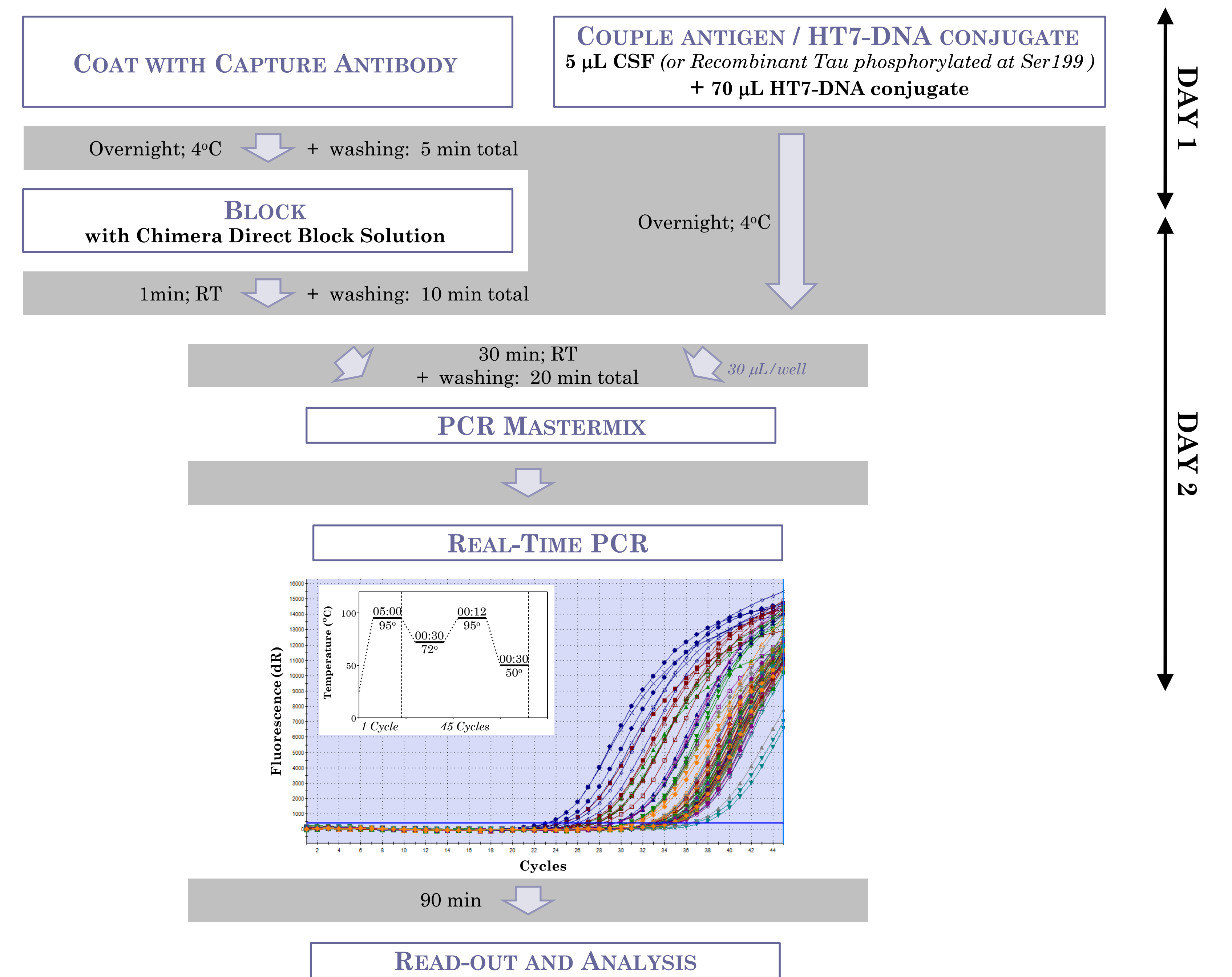
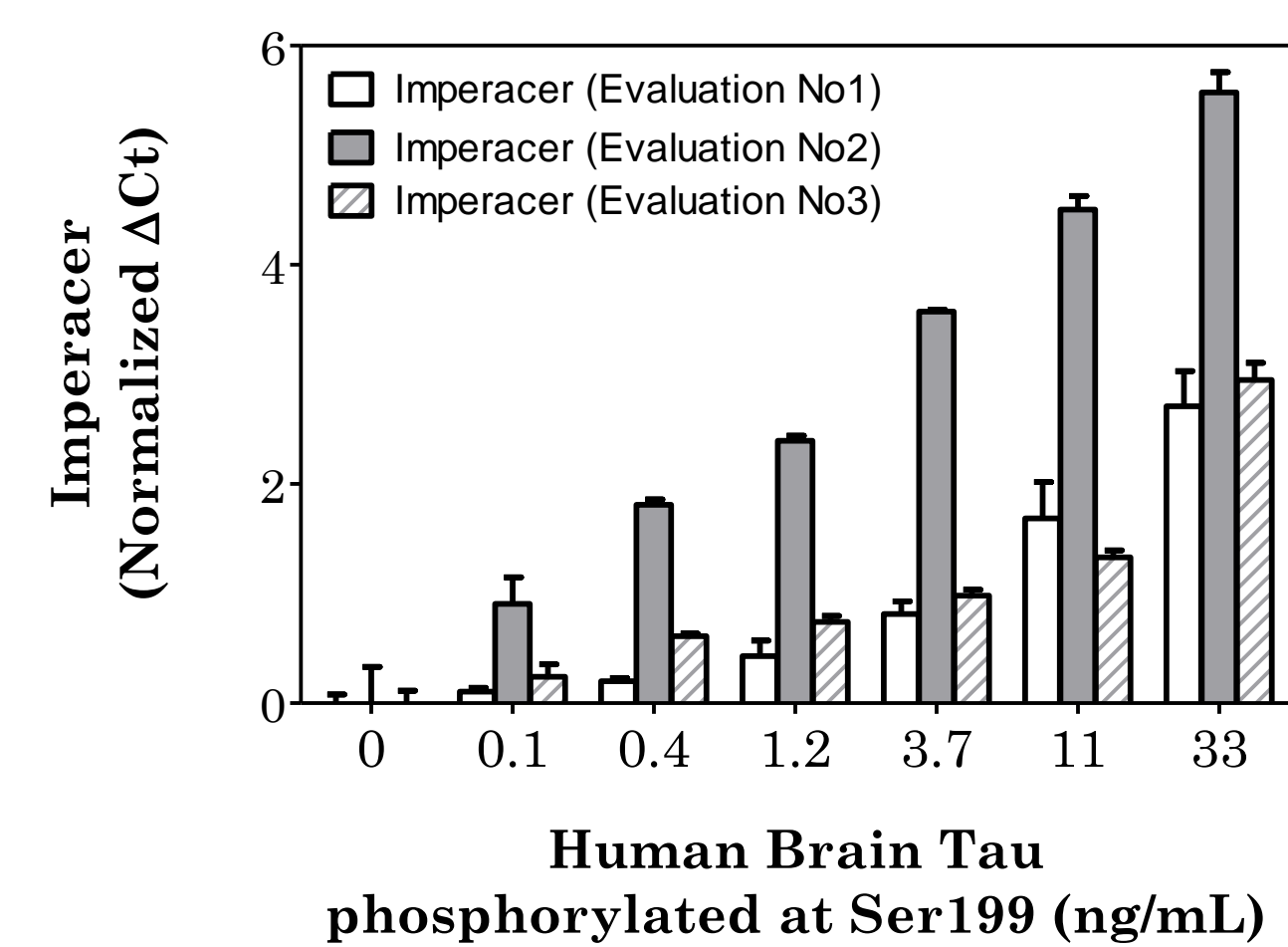


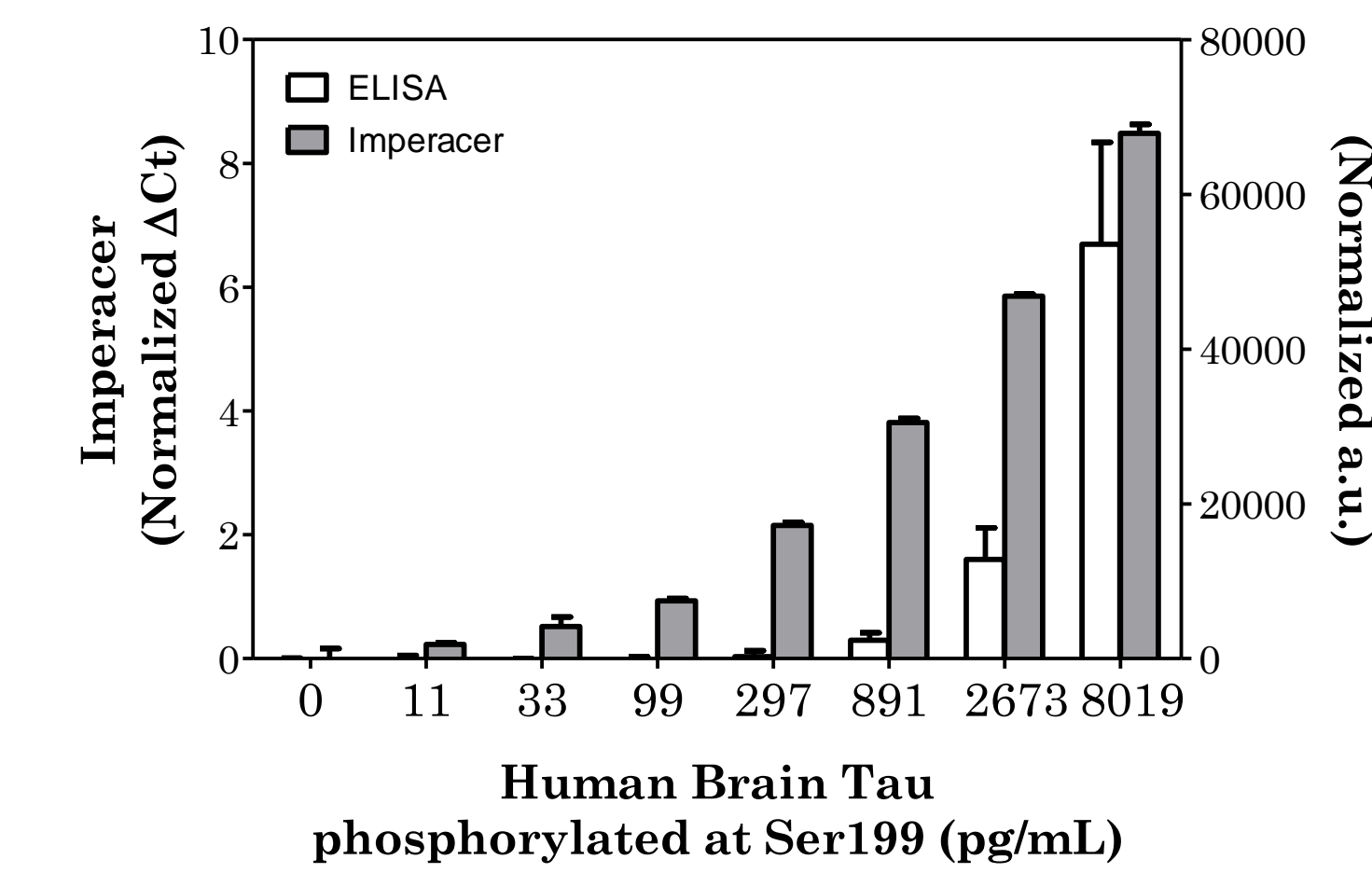
Fig 2. Strategy Optimization



Evaluation	Capture Antibody	Reporter Antibody	Secondary Antibody	Antigen/Reporter Incubation
No1	pSer199	HT7-DNA conjugate	n/a	Sequential
No2	pSer199	HT7-DNA conjugate	n/a	Combined
No3	HT7	pSer199	Rabbit-DNA conjugate	Combined

Data are presented as mean ± SD.

Fig 3. Imperacer versus ELISA



The comparative analysis between Imperacer assay and a conventional sandwich ELISA shows a better sensitivity from our approach.

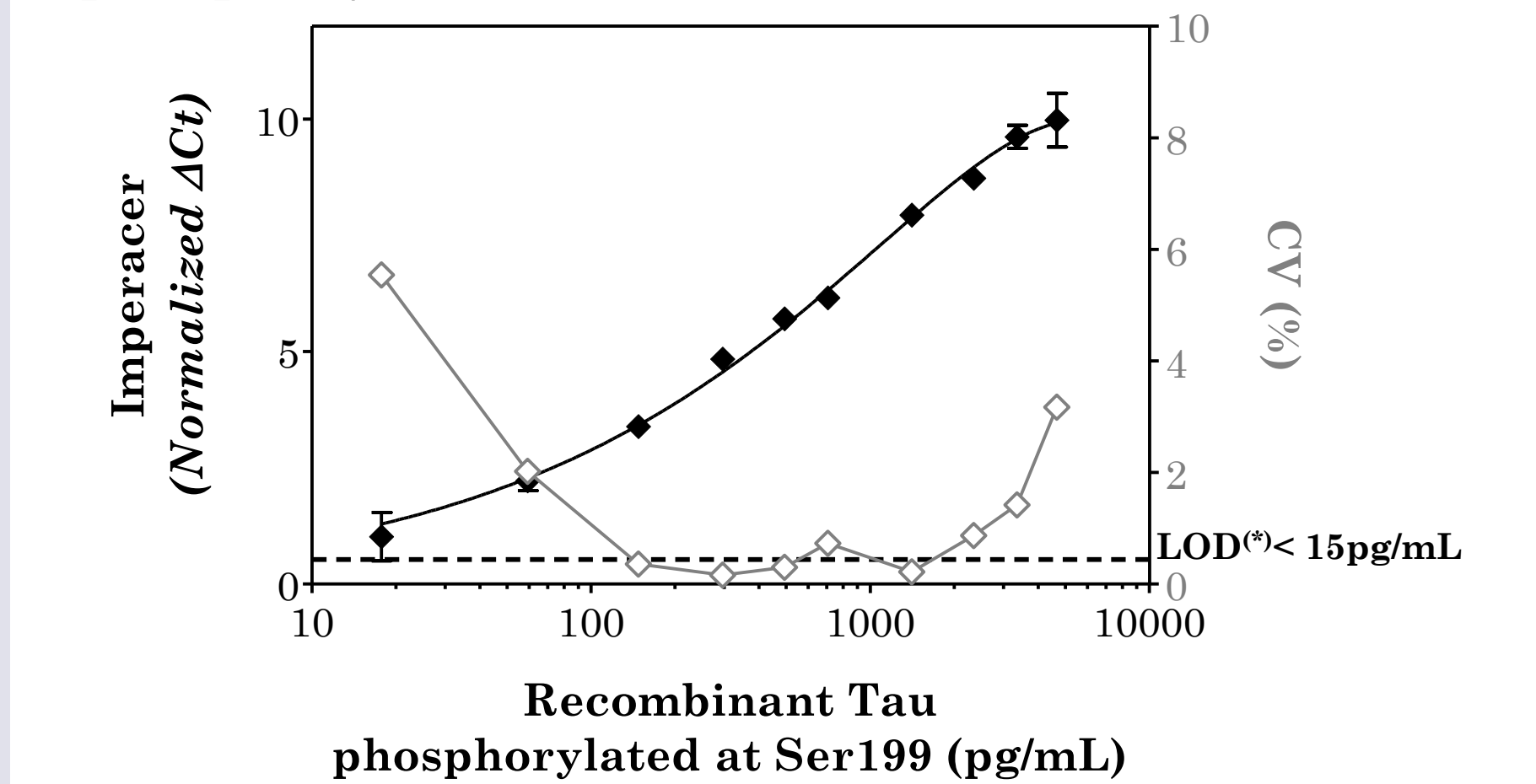
Data are presented as mean ± SD.

## Conclusions

- 1) We have developed an immuno-PCR assay with which pSer199-Tau level can be determined using 2.5µL CSF/assay.
- 2) This technique can be employed similarly to assay levels of other pTau using appropriate capture antibodies.
- 3) We found that phosphorylation of serine 199 is a late event in AD confirming previous results<sup>(1,2)</sup>.

### Analytical Performance

Fig 4. Calibration curve of recombinant Tau phosphorylated at Thr205.

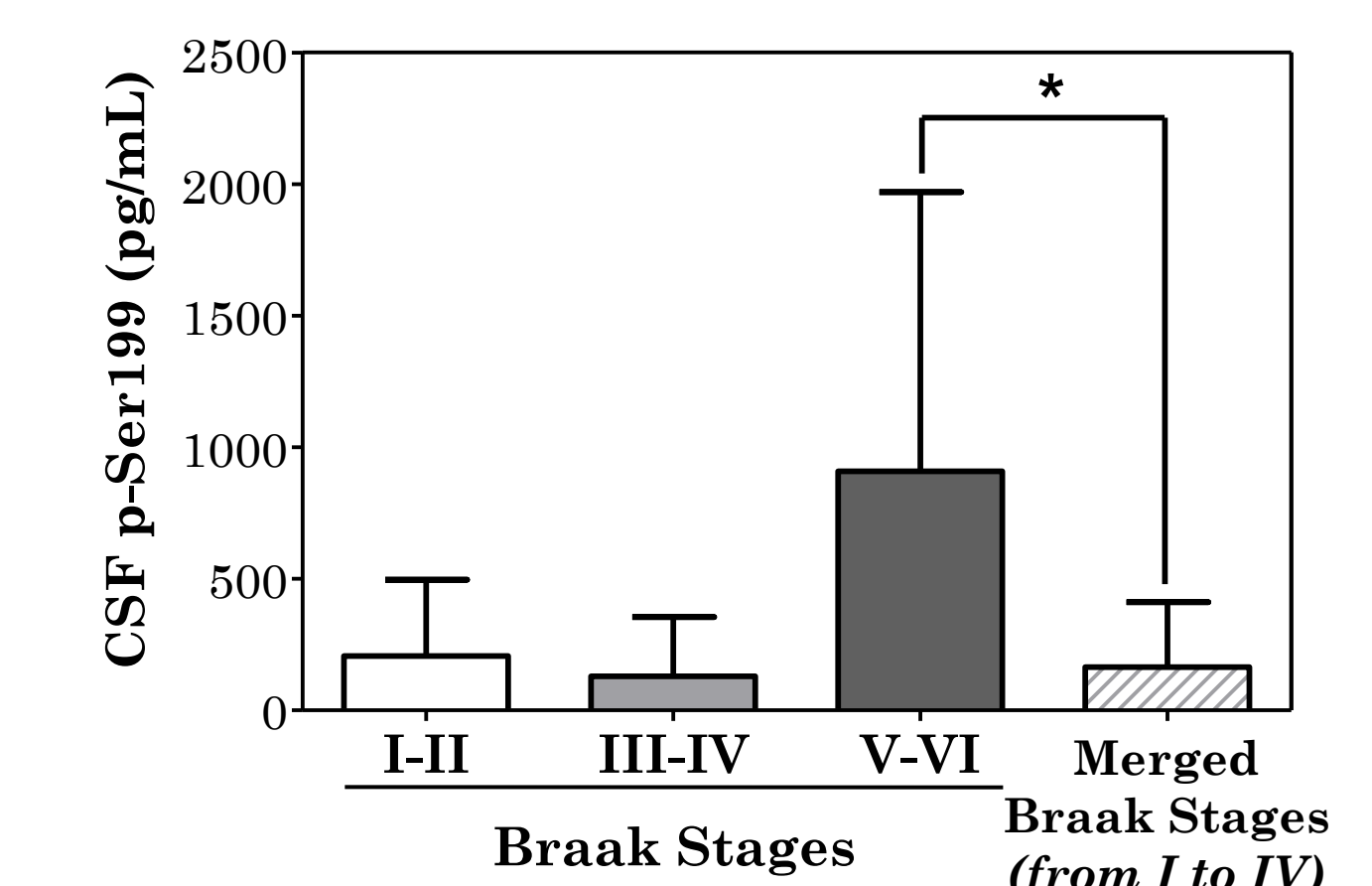


(♦), Imperacer signal from full-length human recombinant tau (Tau<sub>441</sub>) phosphorylated at Ser199 expressed as mean ± SD; [◇], % Coefficient of variation (CV) from intra-assay evaluation (guidelines set by the FDA: %CV < 15%).

(c) LOD, Limit of detection = (3.3 × SD<sub>Negative Control</sub>) + Mean<sub>Negative Control</sub>

### Clinical Performance

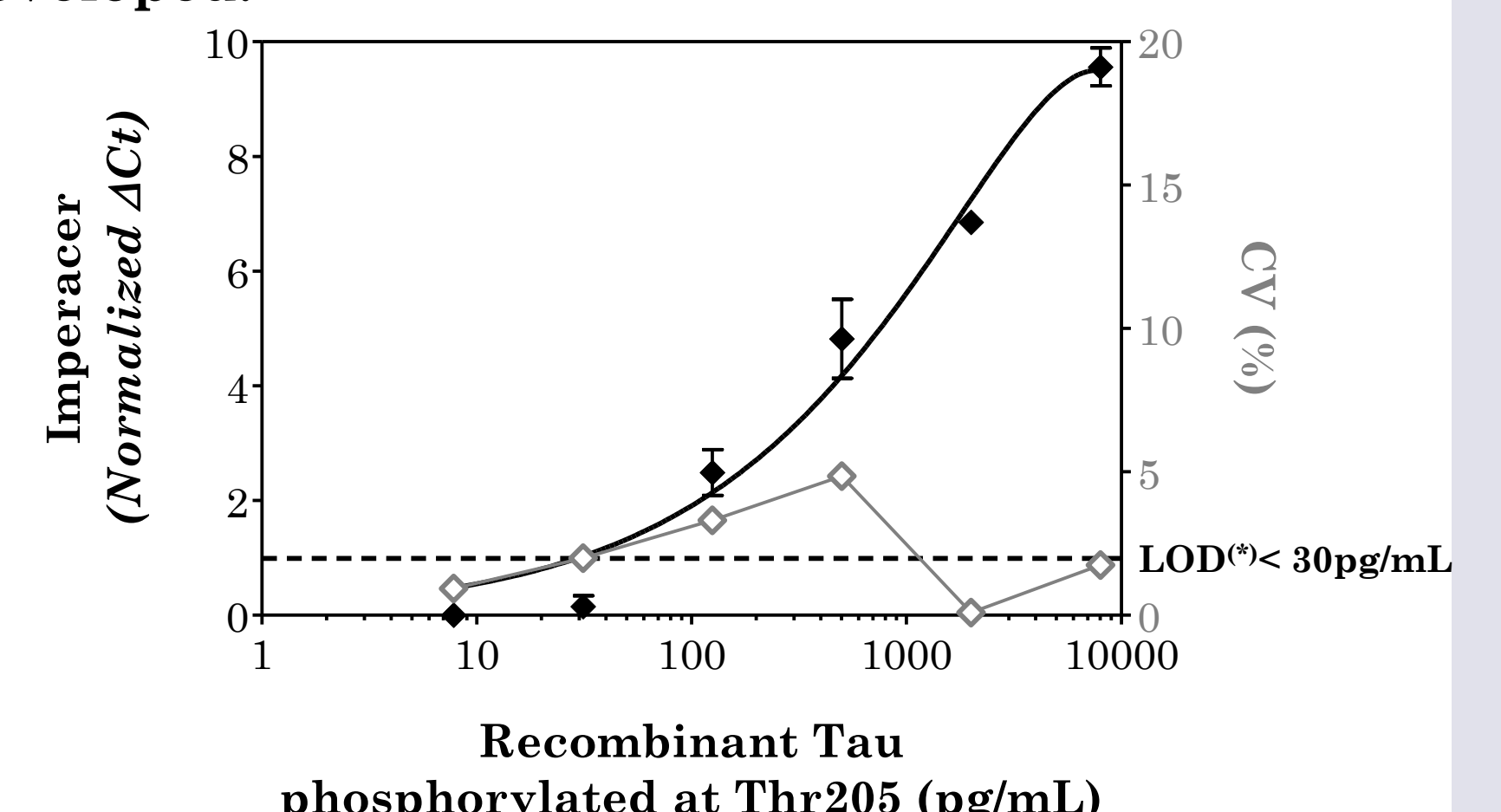
Fig 5. Level of tau phosphorylated at Ser199 in post-mortem CSF.



Data are presented as mean ± SD. No significant difference is observed between I-II, III-IV and V-VI Braak stages groups (Kruskal-Wallis test,  $p = 0.0853$ ). A significant difference is observed once Braak stages from I to IV are merged and compared to V-VI Braak Stages (Mann-Whitney test,  $p < 0.05$ ).

### Perspectives:

Fig 6. Calibration curve of recombinant Tau phosphorylated at Thr205 using qIPCR procedure developed.



(♦), Imperacer signal from full-length human recombinant tau (Tau<sub>441</sub>) phosphorylated at Thr205 expressed as mean ± SD; [◇], % Coefficient of variation (CV) from intra-assay evaluation (guidelines set by the FDA: %CV < 15%).

(c) LOD, Limit of detection = (3.3 × SD<sub>Negative Control</sub>) + Mean<sub>Negative Control</sub>