



ADNI

alzheimer's 
association

Surrogate matrix: BSA-based

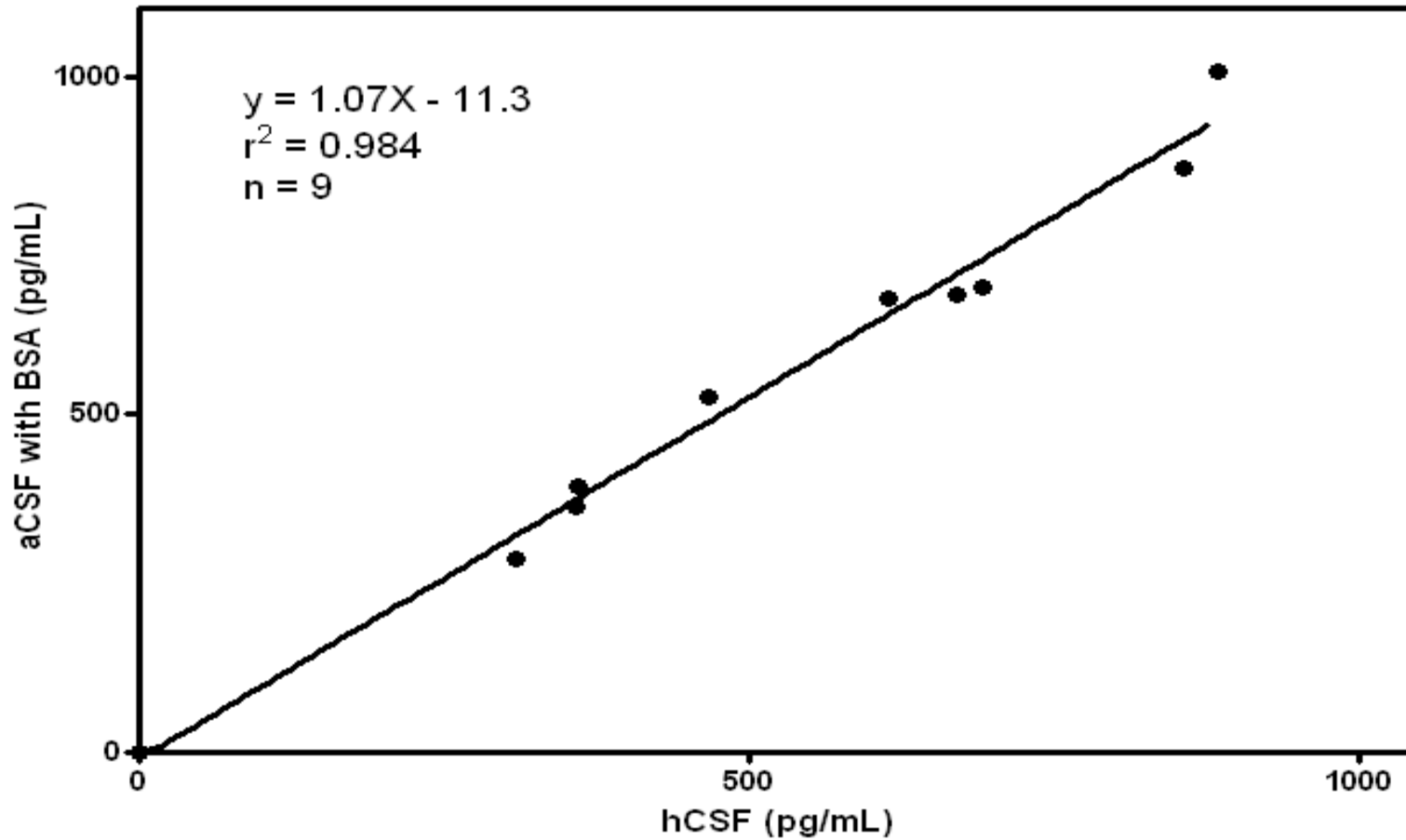


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Protocol for development & validation of the UPenn/ADNI UPLC-srm/tandem mass spectrometry method

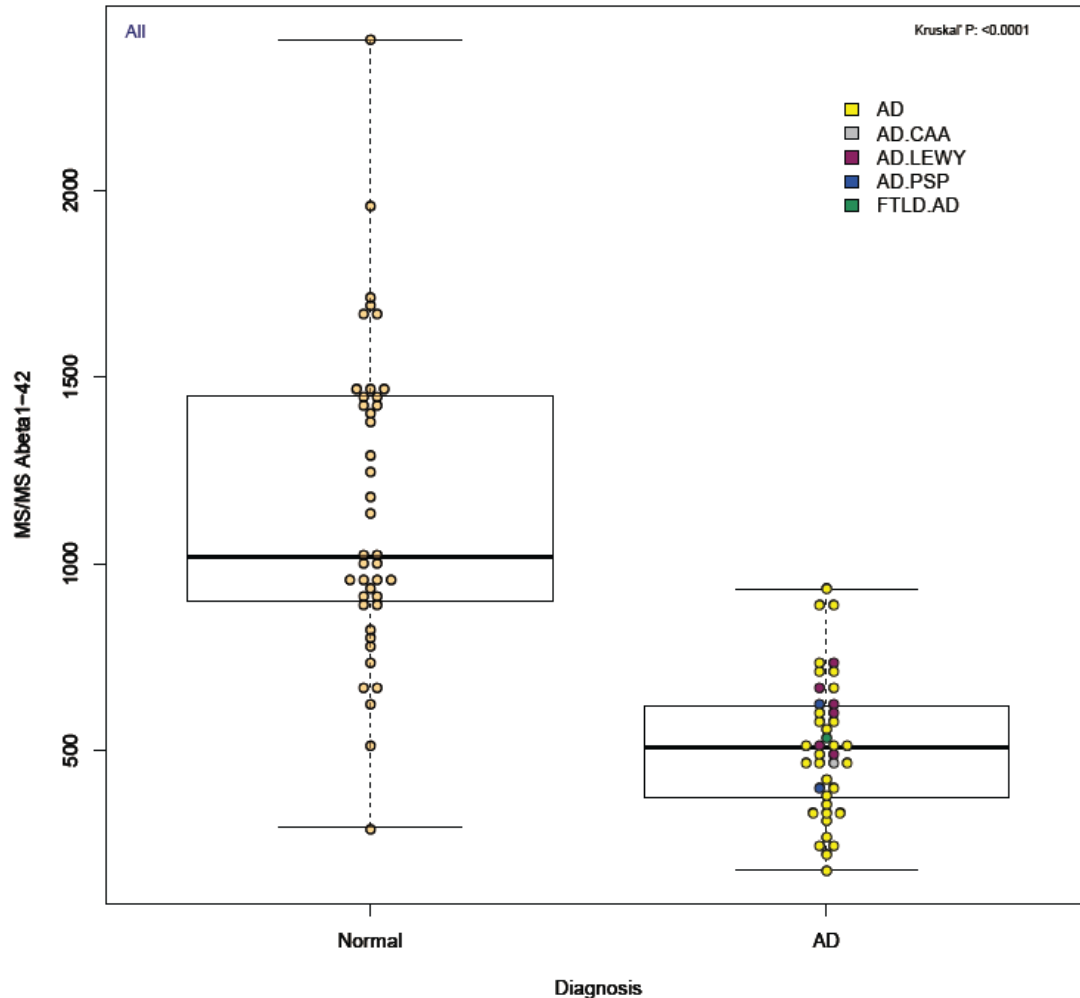
- High conc Guanidine HCl(5 M) to release $A\beta_{1-42}$ from aggregates, oligomers: Lame et al, 2011
- Mixed bed ion exchange 96 well format for 1st step sample cleanup
- Use of a surrogate matrix with equivalent performance to CSF as a calibration matrix: describe all constituents and sources of these
- Use high quality $A\beta_{1-42}$ standard and cross-check performance of several lots of this material; use uniformly N^{15} -labelled IS
- Waters ACUITY 2D HPLC + API 5000 tandem mass spectrometer
- Employ quality controls: *aCSF* (4 mg/mL BSA + electrolytes + $A\beta_{1-42}$) & CSF pools throughout
- Define all major analytical parameters as defined in US FDA Guidance and CLSI guidelines
 - Determine LLOQ & ULOQ
 - Linearity
 - Calibrators' precision and accuracy within- and between-day
 - *aCSF* spiked qc samples (3 spike levels) & 10 CSF pools
 - Spike recoveries from CSF pools and from *aCSF* containing 4 mg/mL BSA
 - Carryover
 - Selectivity (measurement of $A\beta_{1-42}$ in the absence & presence of high concentrations of $A\beta_{1-38}$ and 1-40)
 - Check for “ion suppression” (matrix interference in ionization intensity)
 - Short term stability of calibrators, qc samples & CSF pools (4 hr at room temp)
 - Long-term stability of all qc samples (two CSF pools & 2 *aCSF* spiked controls) over a two year period (ongoing)
- Analysis of AD and control CSF samples in non-ADNI CSF sample aliquots

Comparison of $A\beta_{1-42}$ measured in 9 CSF pools using BSA/aCSF for calibration vs using CSF pools for calibration (using the method of standard addition in these pools)

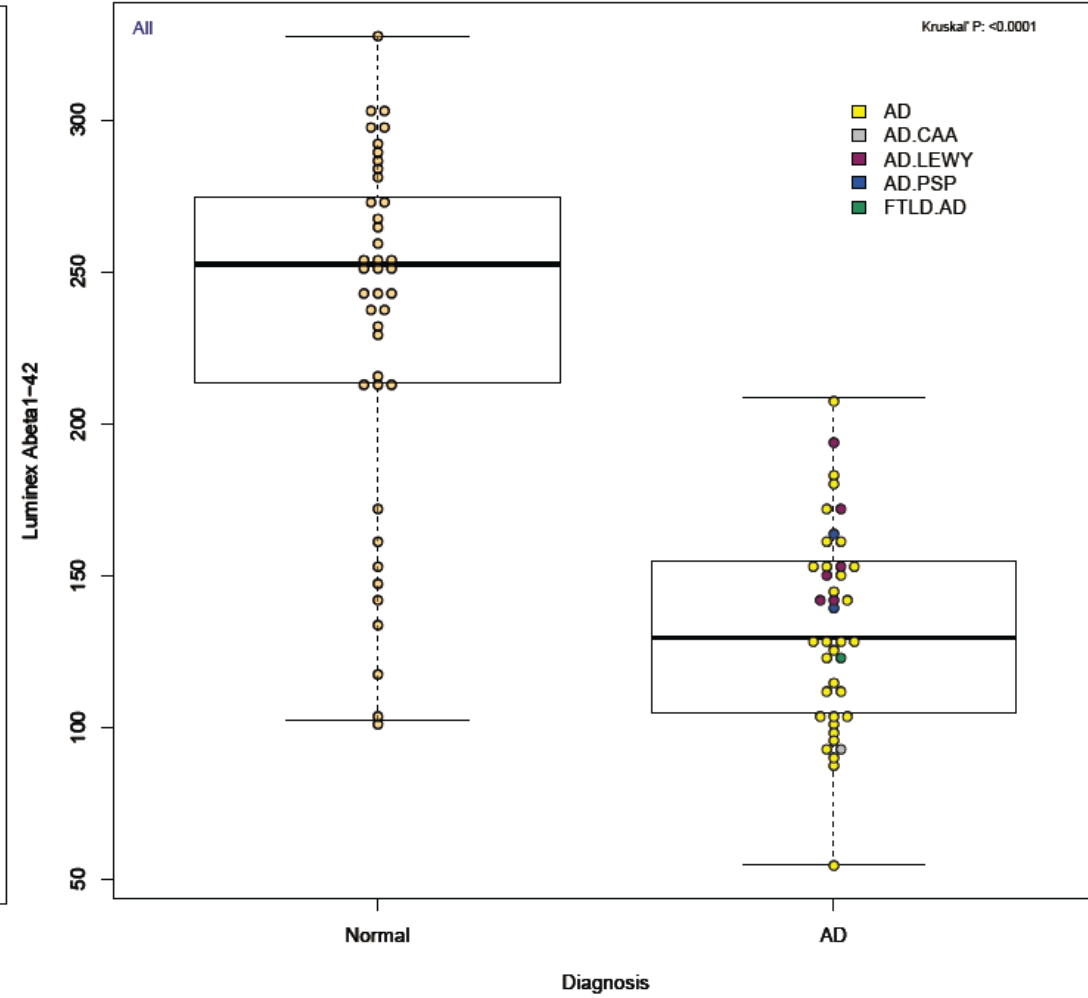


$A\beta_{1-42}$ concentrations measured in 41 autopsy-confirmed AD & 41 age-matched controls

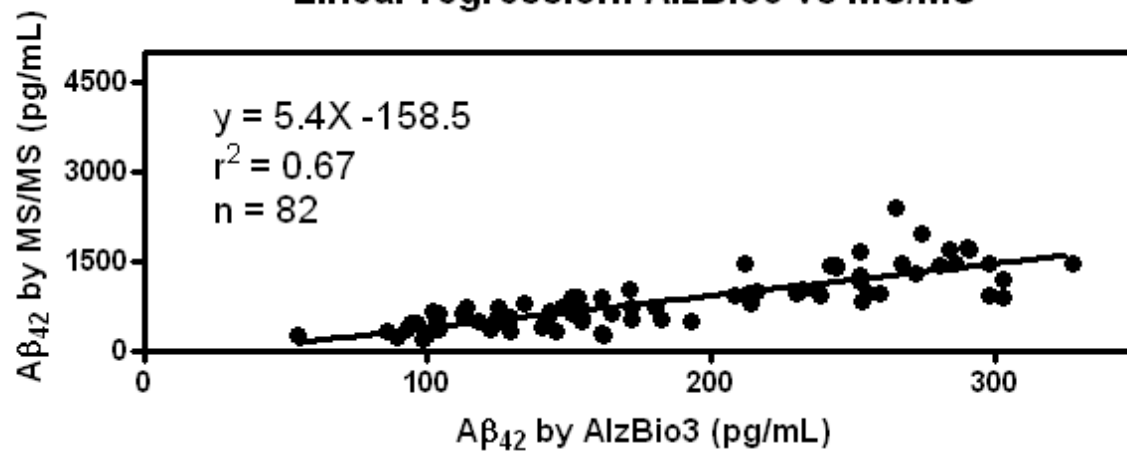
SRM/tandem mass spectrometry



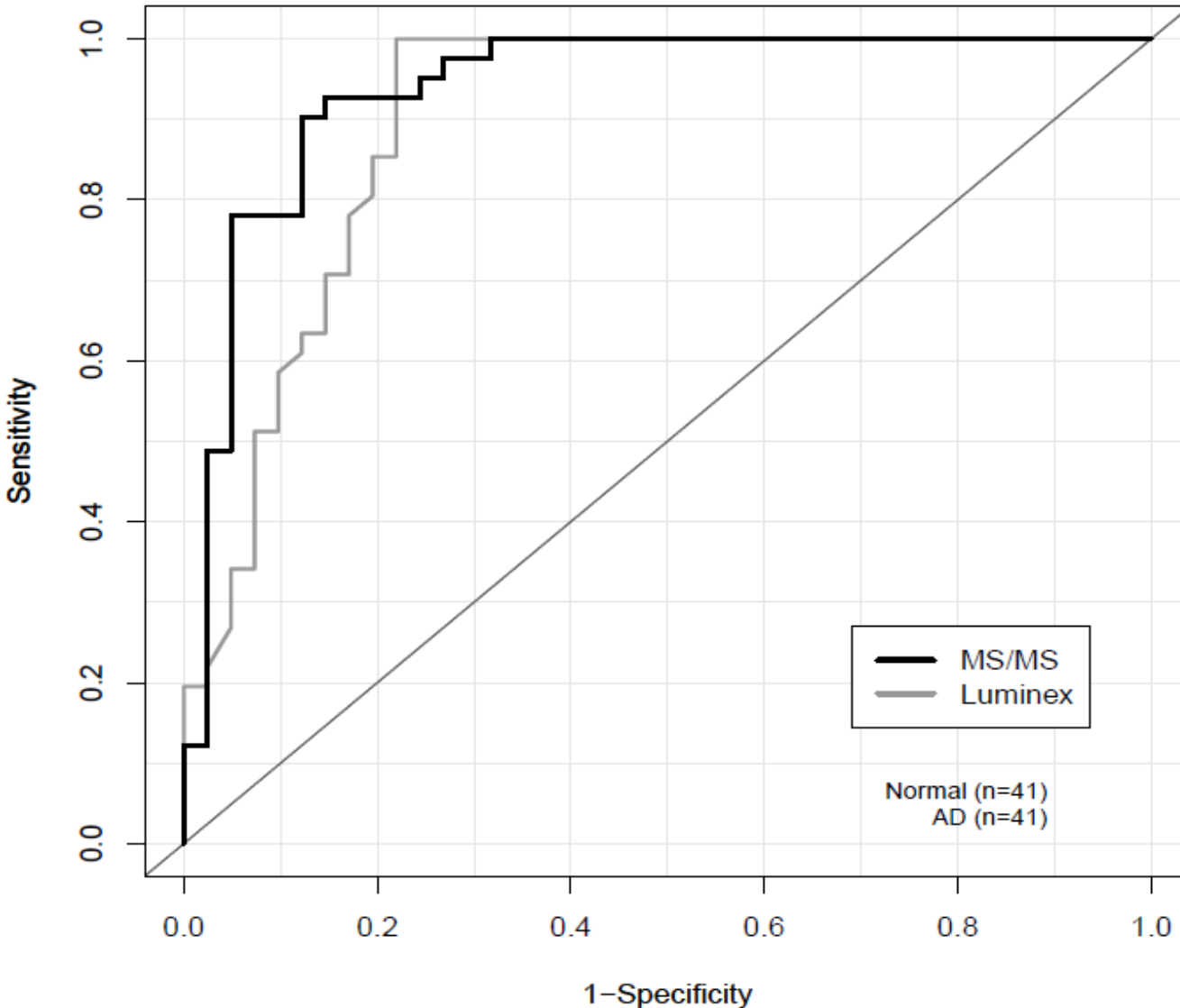
AlzBio3 xMAP™ immunoassay



Linear regression: AlzBio3 vs MS/MS



ROC curve analysis



ROC analyses

Clinical performance using 41 AD, 41 age-matched cog normal controls for the mrm/MSMS method:

Sensitivity: 92.7%
Specificity: 85.4%
PPV: 86.4%
NPV: 92.1%
Test accuracy: 89%
AUC: 0.94

Clinical performance using the same 41 AD and 41 controls for the AlzBio3 Luminex Immunoassay:

Sensitivity: 100%
Specificity: 78%
PPV: 82%
NPV: 100%
Test accuracy: 89%
AUC: 0.90

$p=0.2229$; DeLong's test for correlated AUC's

Interlab precision studies

- Using same method, AlzBio3, same lot of reagents, and replication over 3 runs: 7 center, report in 2011
- With small refinements in the detailed protocol, AlzBio3, replication over 3 runs: 3 centers including Japan ADNI, Innogenetics/Fujirebio, US ADNI

Study protocol summary

Assay: Evaluation on INNO-BIA AlzBio3 and kit STD & Ctrls → 1 fixed batch
(with extra S7 = S6 1/3 diluted in Diluent)

Samples: 3 * 10 CSF (each provided in aliquots of 500 µL)

→ 4 replicates of each sample per run (2 reported values)

Procedure: 3 independent runs (other day) according to unified test procedure (U-SOP)

Plate set up:

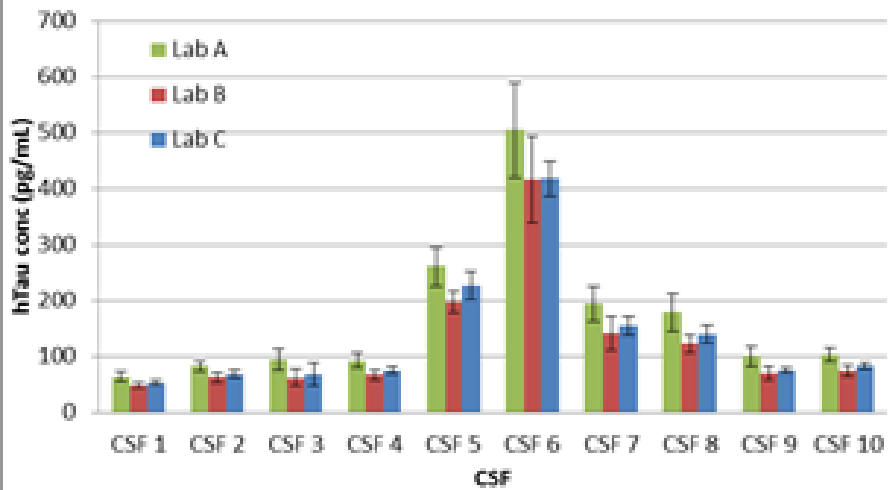
	1	2	3	4	5	6	7	8	9	10	11	12
A	bg		CSF 1		CSF 9		CSF 5					
B	S1		CSF 2		CSF 10		CSF 6					
C	S2		CSF 3		CONTROL A		CSF 7					
D	S3		CSF 4		CONTROL B		CSF 8					
E	S4		CSF 5		CSF 1		CSF 9					
F	S5		CSF 6		CSF 2		CSF 10					
G	S6		CSF 7		CSF 3		CONTROL A					
H	S7		CSF 8		CSF 4		CONTROL B					

Run acceptance criteria:

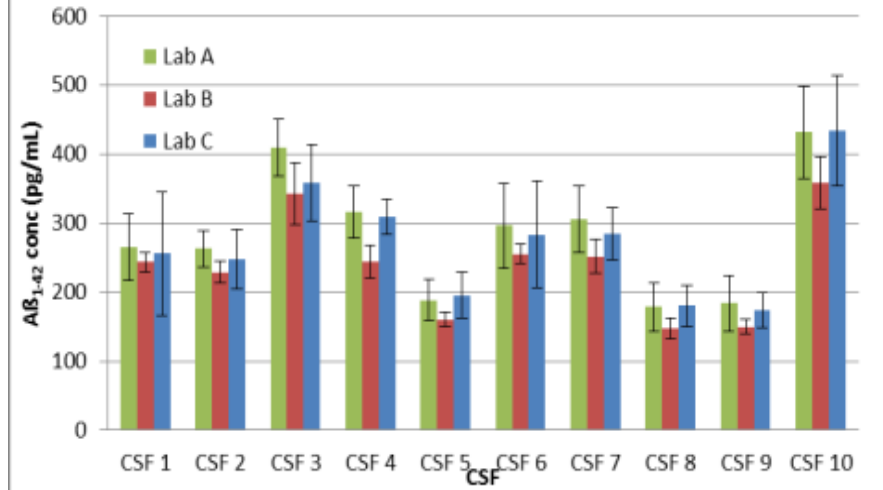
- %CV over duplicate measurements of same sample < 20%
- # beads counted for each analyte ≥ 50

Aim: Evaluation of inter-laboratory variation (reproducibility) of CSF sample concentrations with a U-SOP

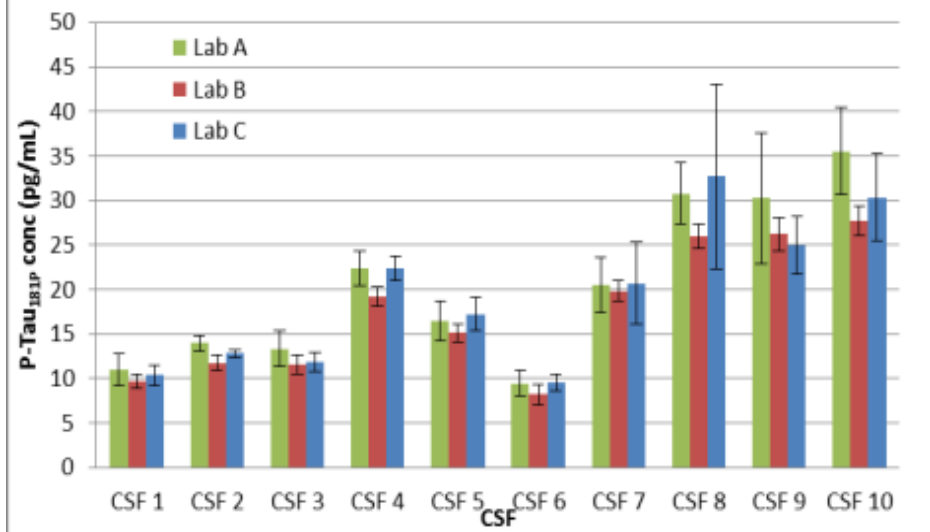
hTau: mean concentration per lab



A β ₁₋₄₂: mean concentration per lab

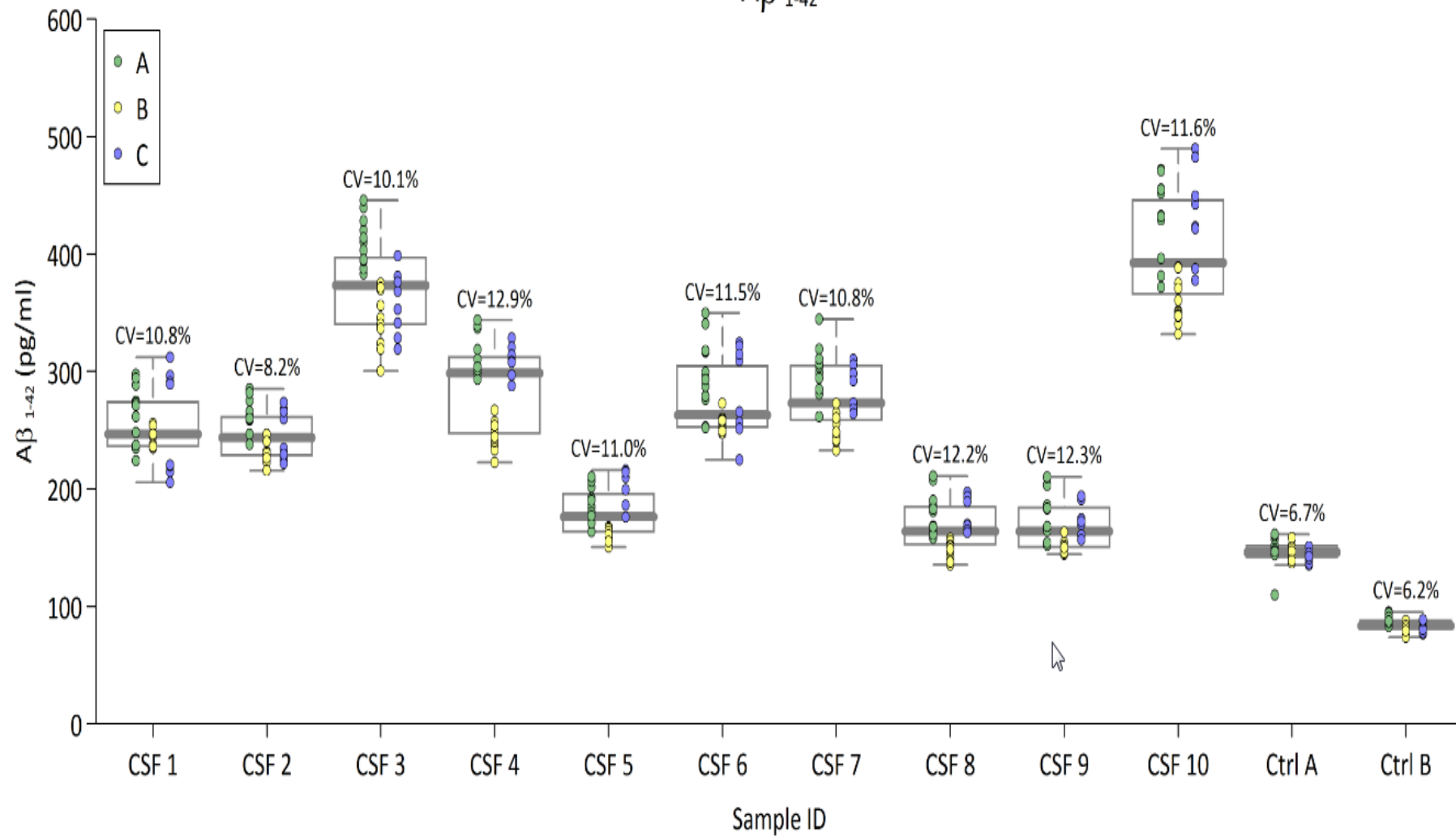


P-Tau_{181P}: mean concentration per lab

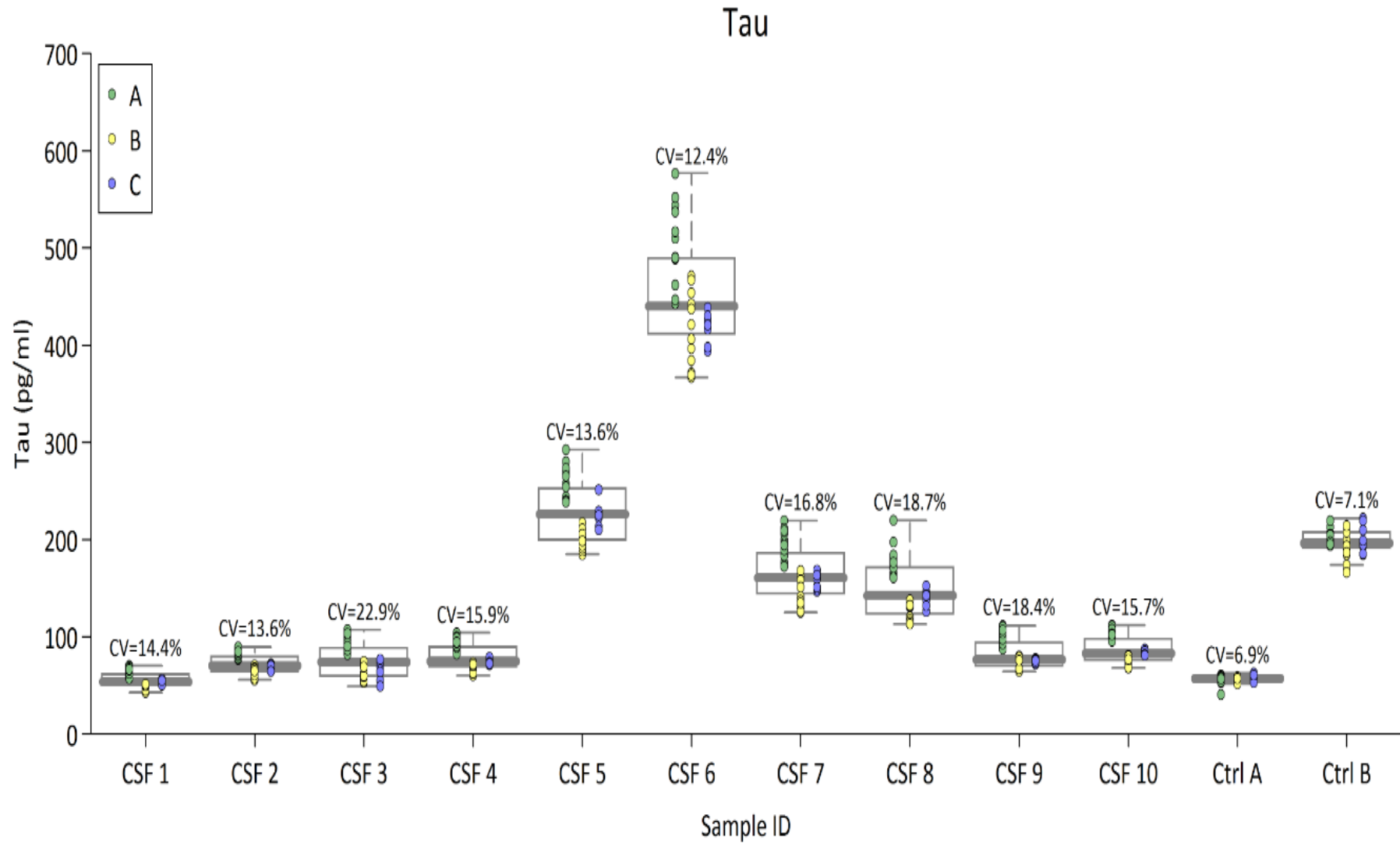


Interlab precision- $A\beta_{1-42}$

$A\beta_{1-42}$

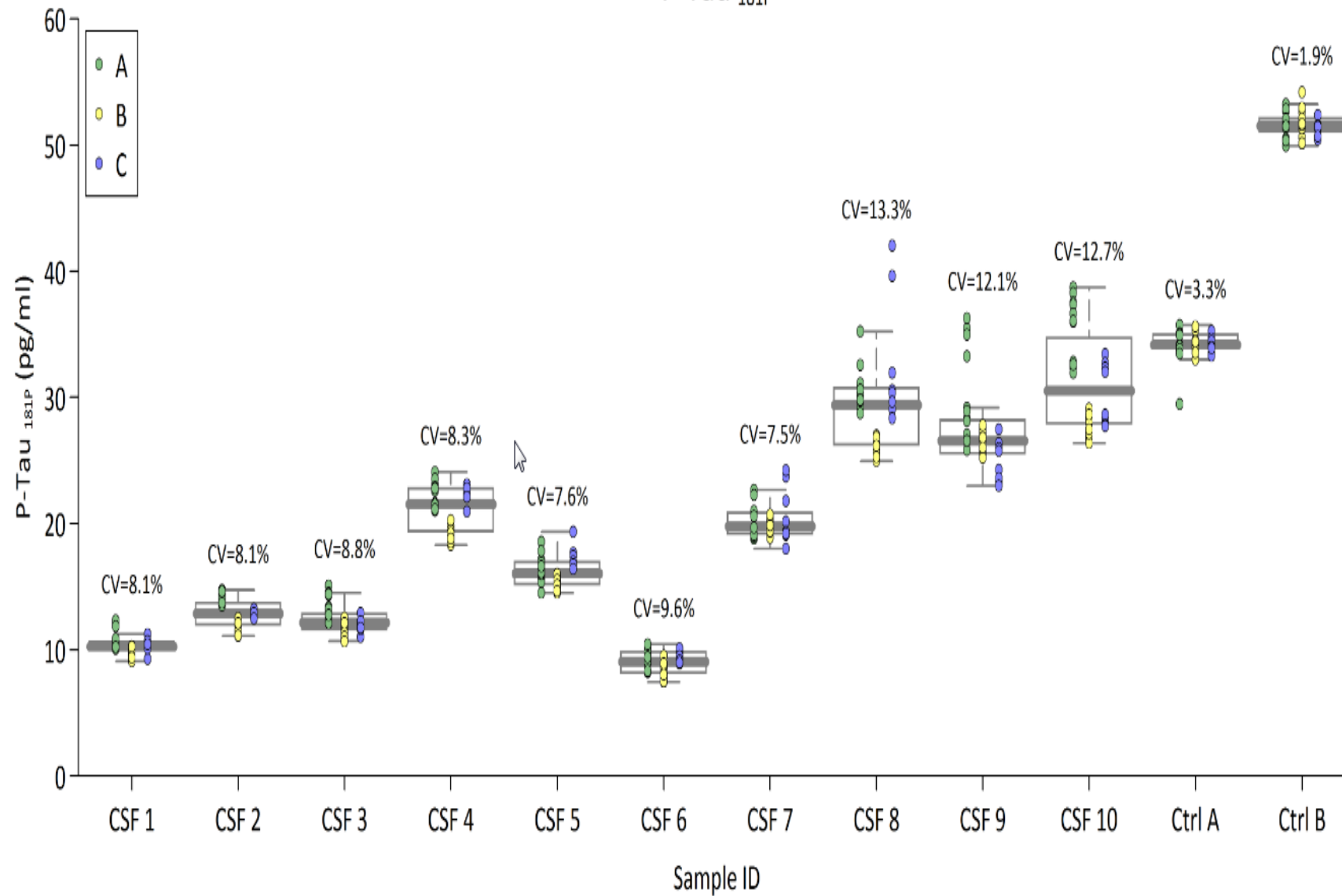


Interlab precision-t-tau



Interlab precision-p-tau₁₈₁

P-Tau_{181P}



Japan ADNI/Fujirebio/NA ADNI interlaboratory precision summary

Analyte	Mean Inter-laboratory variation	Range
t-Tau	16.7%	11.4 – 23.2%
A β ₁₋₄₂	9.3%	4.2 – 13.7%
P-Tau _{181P}	8.2%	2.2 – 12.7%

Conclusions

- Acceptable within- and between center precision performance was obtained
- Development and adherence to a unified SOP protocol is essential for best assessment of precision performance
- Follow-up interlab study planned