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Aβ1-42, t-tau and p-tau181 measurements in 120 PPMI CSF samples using xMAP/Luminex multiplex immunoassay

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Summary

One thousand two hundred and ninety-nine (1,299) CSF samples collected according to the Parkinson's Progression Marker Initiative (PPMI) Research Biomarkers Laboratory Manual were analyzed for the measurement of three CSF biomarkers, amyloid beta (1-42) ($A\beta_{1-42}$), total tau (t-tau) and tau protein phosphorylated at the threonine 181 position (p-tau_{181}) using the multiplex xMAP Luminex platform and Research-Use-Only Innogenetics immunoassay kits (Fujirebio/Innogenetics, Ghent, Belgium). The never before thawed frozen aliquots of CSF samples stored at the PPMI Biorepository Core laboratory (Coriell Institute) or at BioRep repository were transferred to the University of Pennsylvania PPMI Bioanalytics core laboratory and stored at -80 ^{0}C until the day of analysis. Each study subject CSF sample aliquot was labeled with a unique code number. Using aqueous buffer-based standards of three biomarkers, calibration curves were generated for each biomarker. All standards, aqueous controls and CSF samples (including 2 CSF pools for assessment of run quality control) were analyzed in duplicate in each run (total of thirty-eight runs for completion of the analyses) according to SOP LO 04.08 (Luminex method for simultaneous analysis of tau, $A\beta_{1-42}$ and p-tau_{181} in CSF) using Innogenetics Alz Bio3 immunoassay reagents. Included in this report are the following:

1. Summary of precision of duplicates for calibration standards, aqueous quality controls (A and B), CSF pools (55 and 56) and patient CSF samples (Table 1 and Figure 1).
2. Cumulative log-log plots of calibration curves for 7 analysis runs (Figure 2).
3. Quality control data for aqueous quality controls A and B and CSF pools 55 and 56. Measured biomarker concentrations per each of 38 runs are provided for $A\beta_{1-42}$, t-tau and p-tau_{181} and summary mean±SD and %CVs. (Table 2).
4. Repeat testing for results that failed QC criteria for bead counts, duplicate precision or results with median fluorescence below lowest calibrator. (Figures 3A, 3B, 3C & 3D), (Table 3).

Method

For each study subject, CSF was collected in a polypropylene syringe. The first 1-2 mLs of CSF was sent to a local laboratory for routine diagnostic testing and analyzed within 4 hours of collection for cell count, total protein and glucose as described in the Laboratory Manual. The remaining 15-20 mL of CSF was transferred into polypropylene tubes at room temperature, mixed gently, centrifuged at 2000×g for 10 minutes at room temperature and aliquoted into precooled labeled polypropylene sample storage tubes followed by immediate freezing on dry ice. The frozen aliquots of CSF, n=1299, were shipped overnight from Coriell Institute for Medical Research or from the BioRep repository on dry ice to the PPMI Bioanalytics Core laboratory at the Perelman School of Medicine, University of Pennsylvania. Each aliquot was identified with a unique code number.

$A\beta_{1-42}$, t-tau, and p-tau_{181} were measured in each of the never before thawed 1299 CSF PPMI aliquots using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Research-Use-Only Innogenetics INNO-BIA AlzBio3 (Ghent, Belgium) immunoassay kit-based reagents, as described previously (Olsson A et al, 2005; Shaw LM et al., 2011). The lot number for the AlzBio3 immunoassay kits and calibrators used was K231678 and S233319, respectively. In brief, kit reagents included well-characterized capture monoclonal antibodies specific for $A\beta_{1-42}$ (4D7A3), t-tau (AT120), and p-tau_{181} (AT270) chemically bonded to unique sets of color-coded microbeads, and analyte-specific detector antibodies (HT7 for t-tau and p-tau181 and 3D6 for $A\beta_{1-42}$). Using aqueous buffer-based standards of three biomarkers at concentrations ranging from 22 to 2231pg/mL for synthetic $A\beta_{1-42}$, 9 to 1359 pg/mL for recombinant tau, and 4 to 231 pg/mL for a tau synthetic peptide phosphorylated at the threonine 181 position (p-tau_{181}), calibration curves were generated for each biomarker. All standards, two aqueous run controls, two CSF pool quality control samples and PPMI subject CSF samples were analyzed in duplicate. A 5 parameter logistic model with weighting ($1/Y^2$) was used for calibration curve fitting.

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Table 1. Summary of duplicate precision for Standards, QCs, Pools, and PPMI CSF samples.

Duplicate precision results for Standards

Standards	N	Mean [%]	Median [%]	Minimum [%]	Maximum [%]
Tau	260	3.6	2.849	0	18.43
Abeta	263	3.432	2.784	0.08406	12.05
p-Tau	226	1.94	1.311	0	15.31

Duplicate precision results for QCs

QCs	N	Mean [%]	Median [%]	Minimum [%]	Maximum [%]
Tau	76	3.273	2.934	0	9.77
Abeta	76	3.281	3.034	0.03993	10.02
p-Tau	76	1.838	1.414	0.02907	9.634

Duplicate precision results for Pools 55 & 56

Pools **(55&56)**	N	Mean [%]	Median [%]	Minimum [%]	Maximum [%]
Tau	76	2.6637	1.7686	0	13.190120
Abeta	76	2.899	2.7067	0.0175368	9.1085734
p-Tau	76	1.5868	1.3395	0	5.806092

Duplicate precision results for PPMI CSF samples

Samples	N	Mean [%]	Median [%]	Minimum [%]	Maximum [%]
Tau	1268	3.519	2.655	0	23.09
Abeta	1294	3.699	3.055	0.005173	17.9
p-Tau	1293	3.37	2.475	0	18.94

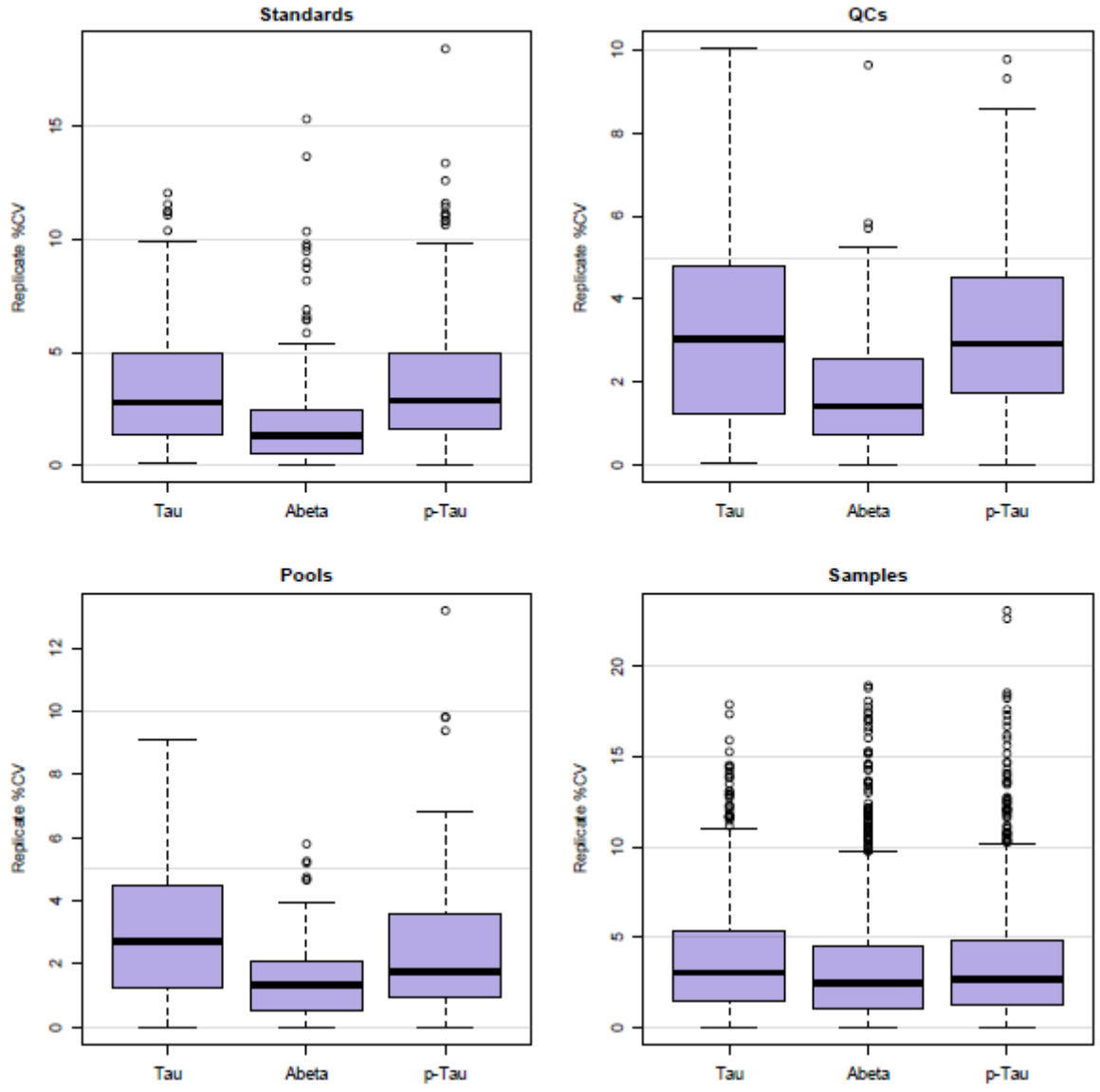
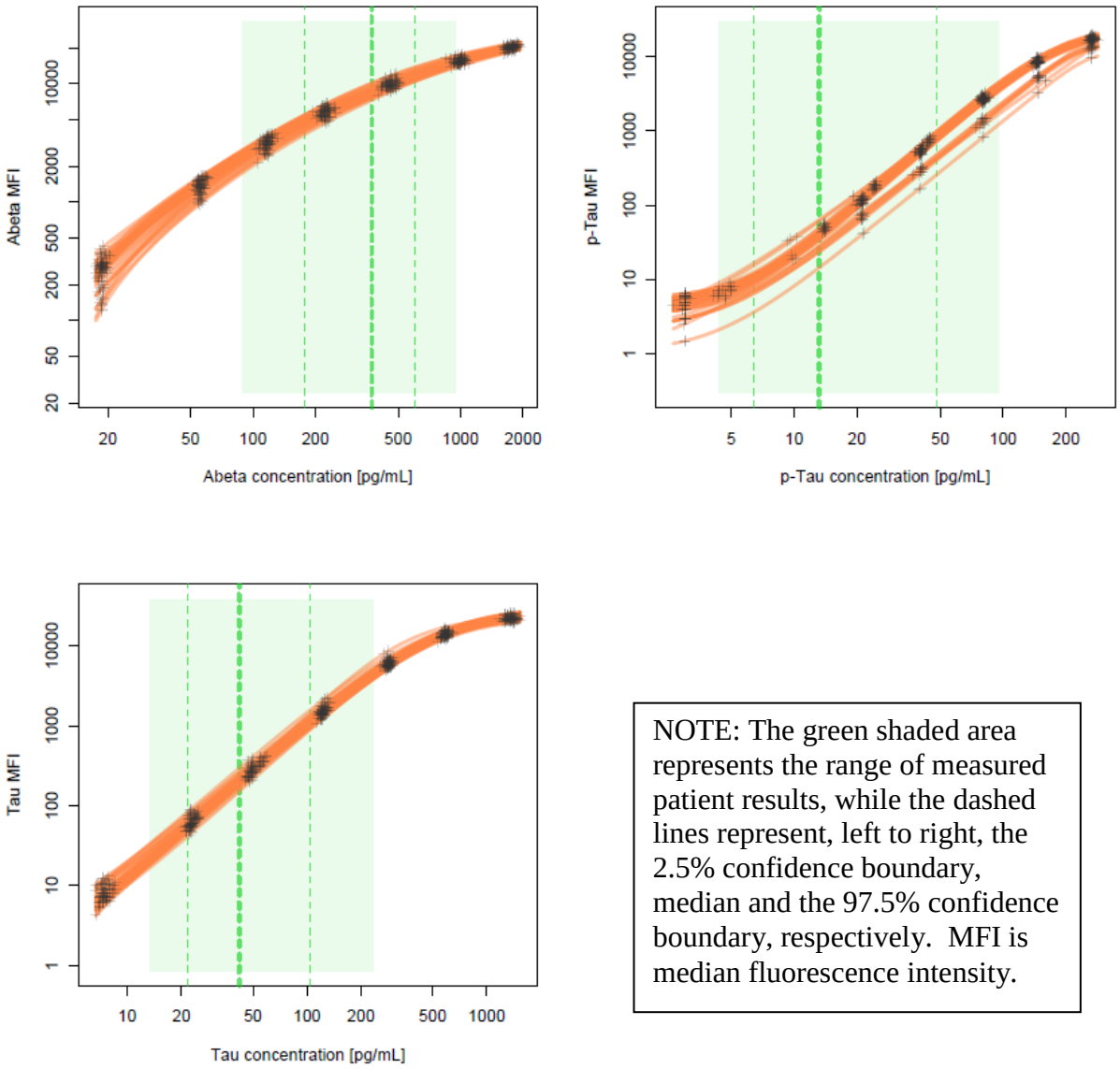


Figure 1 Replicate Precision. Box plots for replicate %CV for CSF $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} for the calibration standards, aqueous controls, CSF pools and patient samples data summarized in Table 1.



NOTE: The green shaded area represents the range of measured patient results, while the dashed lines represent, left to right, the 2.5% confidence boundary, median and the 97.5% confidence boundary, respectively. MFI is median fluorescence intensity.

Figure 2 Calibration Curve-fitting summary

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Table 2. Quality Control data for the 2013 PPMI CSF biomarkers study

QC	Aqueous Controls						CSF QC Pools					
Runs	$A\beta_{1-42}$		t-tau		p-tau_{181}		$A\beta_{1-42}$		t-tau		p-tau_{181}	
	A	B	A	B	A	B	Abnormal Pool 56	Normal Pool 55	Abnormal Pool 56	Normal Pool 55	Abnormal Pool 56	Normal Pool 55
Run 1	145	83	61	208	34	52	182	337	310	54	28	14
Run 2	140	79	63	209	34	53	189	370	324	58	27	17
Run 3	145	82	56	192	34	52	167	326	311	52	26	15
Run 4	147	80	62	206	35	53	189	371	327	53	26	15
Run 5	154	87	60	211	35	54	213	425	343	57	28	16
Run 6	146	82	61	205	34	51	188	384	335	57	27	14
Run 7	154	82	57	209	34	51	167	348	328	53	28	15
Run 8	139	85	64	193	34	50	178	351	315	54	28	15
Run 9	159	92	59	214	35	51	190	376	315	57	24	17
Run 10	158	80	53	197	36	51	187	371	340	58	28	16
Run 11	152	84	66	178	30	52	194	403	333	60	28	17
Run 12	153	80	70	197	30	52	217	397	370	59	28	17
Run 13	155	80	70	192	30	52	153	443	310	64	27	16
Run 14	139	78	72	189	29	51	169	342	323	62	24	18
Run 15	153	78	67	189	26	52	169	333	343	61	30	12
Run 16	147	82	74	187	30	52	170	374	343	59	29	16
Run 17	146	82	71	186	29	49	198	356	340	59	29	16
Run 18	146	82	70	197	29	52	168	380	323	61	28	16
Run 19	162	84	67	189	30	52	167	353	318	59	29	16
Run 20	139	76	68	183	30	52	167	364	327	59	27	16
Run 21	154	78	70	190	28	52	195	368	348	59	29	16
Run 22	153	73	72	189	31	52	179	393	329	63	27	18
Run 23	154	84	69	191	30	52	192	370	316	57	25	17
Run 24	150	78	69	188	28	53	157	427	349	57	26	14
Run 25	145	81	71	174	29	50	195	340	326	62	29	17
Run 26	151	76	72	184	30	51	187	422	344	60	31	16
Run 27	159	88	71	180	30	54	180	381	328	64	30	15
Run 28	159	83	68	195	30	54	164	351	327	64	31	16
Run 29	154	83	70	192	31	55	180	335	280	58	30	16
Run 30	163	87	68	185	31	55	182	359	354	48	27	16
Run 31	159	89	72	196	27	54	194	365	328	59	28	12
Run 32	148	84	74	194	30	54	207	377	302	60	30	15
Run 33	163	82	79	202	31	54	164	389	266	58	26	16
Run 34	161	82	77	193	30	52	218	329	348	52	28	15
Run 35	153	85	74	208	31	54	174	467	350	64	31	16
Run 36	157	91	65	199	30	55	175	362	340	64	31	16
Run 37	143	82	72	192	29	56	163	356	360	56	30	16
Run 38	153	84	70	198	29	52	190	313	315	65	24	16
Mean±SD	152±7	82±4	68±6	194±10	31±2.5	52±1.6	182±16	371±33	329±20	59±3.9	28±1.9	16±1.3
Mean%CV	**4.6**	**4.9**	**8.6**	**4.9**	**7.9**	**3.0**	**8.8**	**9.0**	**6.2**	**6.7**	**7.0**	**8.2**

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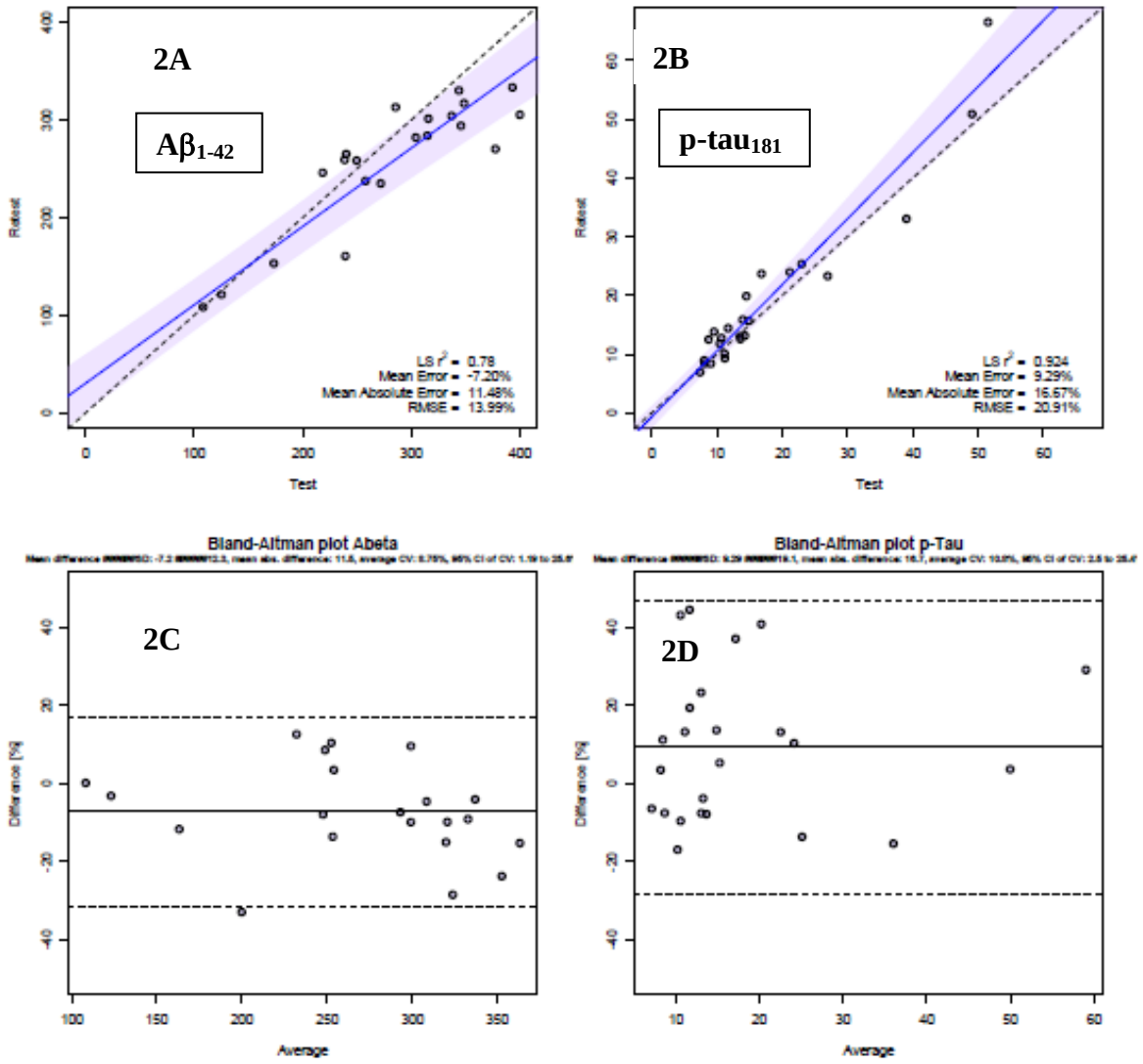
Repeat testing for results that failed QC criteria for bead counts, duplicate precision or results with median fluorescence intensity below that of the lowest calibrator.

Figure 2A & 2B: Deming regression plots for $A\beta_{1-42}$ and p-tau_{181}, respectively, for the samples that failed the bead count criterion for t-tau and were repeat tested in a later run.
Figure 2C & 2D: Bland-Altman plots for $A\beta_{1-42}$ and p-tau_{181}, respectively. All aliquot samples were re-frozen following initial testing, and re-thawed on the day of repeat testing.



Tables 3A & 3B. Missing data summary tables.

Table 3A. PPMI CSF samples with bead counts <50

	Initial run fail	Repeat run fail
t-tau	13	8
$A\beta_{1-42}$	3	0
p-tau	4	0

Table 3B. PPMI CSF samples with Median Fluorescence < lowest calibrator.

	Initial run fail	Repeat run fail
t-tau	0	--
$A\beta_{1-42}$	0	--
p-tau	3	--

References

1. Olsson A, Vanderstichele H, Andreasen N, et al. Simultaneous measurement of β-amyloid(1-42), total tau and phosphorylated tau (thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005;51:336 –345
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