PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

$A\beta_{1-42}$, t-tau and p-tau_{181} measurements in 120 PPMI CSF samples using xMAP/Luminex multiplex immunoassay

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Summary

One hundred and twenty 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 research-use-only multiplex xMAP Luminex platform and Innogenetics immunoassay kits (Innogenetics/Fujirebio, Ghent, Belgium). The never before thawed frozen aliquots of CSF samples stored at the PPMI Biorepository Core laboratory (Coriell Institute) were transferred to the University of Pennsylvania PPMI Bioanalytics core 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 four runs for completion of the analyses). The % CV for replicates for the three biomarkers in two aqueous controls and two CSF pools are summarized in the table below. The %CV values for the duplicate analyses for PPMI study subject CSF samples were below 25%, except for t-tau (6 samples) showed over 25 % CV (26.9 – 29.0 %), and $A\beta_{1-42}$ and p-tau_{181} in 1 sample and t-tau in 2 samples showed low bead counts (< 50). These data are included in the report but we point these out as the results in these cases are not as precise as the remainder of the data.

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 pre-cooled labeled polypropylene sample storage tubes followed by immediate freezing on dry ice. The frozen aliquots of CSF, n=120, were shipped overnight from Coriell Institute for Medical Research 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 120 CSF PPMI

aliquots using the research-use-only multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics INNO-BIA AlzBio3 (Ghent, Belgium) immunoassay kit-based reagents, as described previously (Olsson A et al, 2005; Shaw LM et al., 2011). 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-tau_{181} and 3D6 for $A\beta_{1-42}$). Using aqueous buffer-based standards of three biomarkers at concentrations ranging from 22 to 2231 pg/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, controls and CSF samples (including 2 CSF pools for run validation) were analyzed in duplicate. A 5 parameter logistic model with weighting($1/Y^2$) was used for calibration curve fitting. The lot numbers for the AlzBio3 immunoassay kits and calibration standards used in this study were: 215369 and 213775, respectively.

Table 1. QC data for the PPMI CSF biomarkers study

Aqueous Control		**Run1**	**Run2**	**Run3**	**Run4**	**Mean ± S.D.**	**Mean %CV**
$A\beta_{1-42}$	A	159	170	161	157	162 ± 5.8	**3.60**
	B	75	74	69	76	73 ± 2.9	**3.99**
t-tau	A	103	90	94	87	93 ± 7.0	**7.45**
	B	192	202	197	185	194 ± 7.3	**3.77**
p-tau_{181}	A	34	35	33	33	34 ± 1.2	**3.67**
	B	44	47	42	44	44 ± 2.3	**5.17**
CSF pools		**Run1**	**Run2**	**Run3**	**Run4**	**Mean ± S.D.**	**Mean %CV**
$A\beta_{1-42}$	C01	187	216	215	179	195 ± 18.9	**9.67**
	D11	199	181	205	189	193 ± 10.4	**5.37**
t-tau	C01	153	170	158	158	159 ± 8.1	**5.09**
	D11	52	50	54	45	51 ± 3.9	**7.69**
p-tau_{181}	C01	23	24	23	23	23 ± 0.8	**3.29**
	D11	15	16	16	16	16 ± 0.4	**2.72**

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

2. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol 2011;121:597-609.

3. Parkinson's Progression Marker Initiative Research Biomarkers Laboratory Manual (Biologic Manual). http://www.ppmi-info.org/wp-content/uploads/2011/05/PPMI-Biologics-Manual-April-2011-FINAL.pdf

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