

# **ADNI 2: Fourth batch analyses of CSF biomarkers**

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### Summary

A total of 230 (112 BASELINE plus 2 yr longitudinal CSF aliquot samples, five(5) 24 month samples and 1 BASELINE only CSF sample) never before thawed aliquots of ADNI 2 CSF samples collected between 1/20/2011 and 12/10/2014, (collection dates provided in UPENNBIOMK8 .CSV file), plus 11 of these aliquots were repeat tested in a subsequent run. All paired longitudinal samples were included in the same testing run on the same 96 well analytical plate. One or two of these "re-test" aliquots were included in each run subsequent to the first run. Each calibration standard sample, quality control sample and ADNI study subject sample were run in duplicate according to the manufacturer's instructions. Each test result is the mean value of the duplicates. The attached "ADNI GO and 2 CSF:2015" report provides details for the analyses including calibrator and quality control samples performance, test/re-test performance and the raw data for these analyses. The accompanying ADNI 2 2015 CSF A $\beta_{1-42}$ , t-tau and p-tau<sub>181</sub> dataset in .csv file format (UPENNBIOMK8) provides the final set of results following rescaling (Transformation) based on 2013 BASELINE ADNI results, according to the procedure described below, for the 2015 ADNI GO and II CSF sample analyses. In addition, the UPENNBIOMK8 data file includes the RAW untransformed data.

### Method

The xMAP Luminex platform and Fujirebio AlzBio3 immunoassay kits were used following the SOP in place at the UPenn/ADNI Biomarker Laboratory, according to the kit manufacturer's instructions and as described in previous publications (1-4). Analyses were performed in a series of 10 runs using a 96 well plate format, over the time period of December 5, 2014 through February 13, 2015. Acceptance criteria as documented in the UPenn/ADNI Biomarker Laboratory SOP were followed for these analyses.

Each of the 10 analytical runs met acceptance criteria for calibrator precision and accuracy (back calculated concentration result vs nominal concentration result) and quality control results were within stated limits (detailed data in "ADNI GO and 2 CSF report: 2015"). Individual sample results were acceptable in all cases except for a single t-tau result, where noted, and that was reported as "NA" in the .CSV file "UPENNBIOMK8".

In order to assure cross-sectional comparability of results between these ADNI GO + ADNI 2 subject CSF samples and the earlier 2013 BASELINE CSF biomarker results (UPENNBIOMK6 dataset), assessment of the concentrations of A $\beta_{1-42}$ , t-tau and p-tau<sub>181</sub> were performed in a set of 99 never before thawed ADNI GO and II patient BASELINE CSF aliquots ("sister" pristine aliquots had previously been run in the 2013 batch runs (reported in UPENNBIOMK6) and new

pristine aliquots were included in the series of 112 longitudinal sample sets from ADNI GO and II subjects run in the 2015 batch run). Linear regression analyses (Passing-Bablok) were performed for  $A\beta_{1-42}$ , t-tau and p-tau<sub>181</sub> comparing RAW CSF concentration results obtained in 2015 with those obtained in the analyses performed in 2013(referred in Figure 2 as "2013T") transformed to 2007 BASELINE as described in UPENNBIOMK5) (see Figure 2 in this Methods report). Correlation results were obtained ( $R^2$  values of 0.905, 0.902 and 0.81 for  $A\beta_1$ . <sub>42</sub>, t-tau and p-tau<sub>181</sub>, respectively; for A $\beta_{1-42}$  the slope value is 1.51 and y-intercept value is -37.8 pg/mL, for t-tau the slope value is 0.788 and y-intercept is 6.46 pg/mL and for p-tau<sub>181</sub> the slope is 0.41 and v-intercept is 7.64 as summarized in Figure 2). The slope and intercept values were then used to bridge between the 2013 data and the current 2015 CSF concentrations. This was accomplished by solving the equation, X = (Y-b)/m [X is the rescaled (Transformed) 2013 result; Y is the raw 2015 result; m is the slope of the regression analysis and b is the Y intercept value of the regression analysis summarized in Figure 2]. For studies that use 2015 ADNI GO + ADNI 2 CSF biomarker concentration results, we recommend the use of the transformed results. As noted in the Summary the raw data can be found in the UPENNBIOMK8 dataset csv file and is also presented in the analytical report entitled "ADNI GO and 2 CSF:2015" report.

**Figure 1.** Linear regression (Passing-Bablok) analysis plots for 2015 CSF  $A\beta_{1-42}$ , t-tau and p-tau<sub>181</sub> data rescaled [Transformed, (T)] to 2013 T baseline data vs the 2013 results for those samples, rescaled [Transformed, (T)] to the 2007 BASELINE dataset. The plot on the right side of each linear regression plot is a Bland-Altman bias plot of the %difference between the 2015(T) result and the corresponding 2013 (T) result.

Performance assessment for AlzBio3 reagents: 2015T vs 2012/13T



# **Α**β<sub>1-42</sub>:









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In Figure 1 A $\beta_{1-42}$  2013 (T), t-tau 2013 (T) and p-tau<sub>181</sub> 2013 (T) data are ADNI 2013 BASELINE Transformed CSF results on 99 subjects, using the Research Use Only Innogenetics AlzBio3 xMAP immunoassay. Abeta 2015 (T) are never before analyzed replicate CSF aliquots (continuously stored at -80 °C) from the 99 subjects whose values were rescaled(Transformed, T) as discussed in the paragraph above. The analyses done in 2013, done as one batch in 2013 and transformed as described in the document "*ADNI GO and ADNI 2 First batch analyses of CSF biomarkers: revised anchoring to 2007 ADNI 1 BASELINE dataset procedure"*, included ADNI 1 BASELINE pristine CSF samples. The analyses done in 2013 were done as one batch (with different lots of reagents and calibrators, than the lot# used in the 2007 analyses), using Fujirebio/Innogenetics AlzBio3 xMAP immunoassay reagents. Shaded areas are the 95% CI for the regression fit line. R<sup>2</sup> values are from least squares analyses of the data. Slope and intercept values determined using Passing-Bablok linear regression. The P-B linear regression parameters for each biomarker for the 2015 (T) vs 2013 (T) results are summarized in Table 1 below.

Table 1. . P-B linear regression equation parameters defined by anchoring 2014 analyses of one hundred & twelve (99) ADNI GO/2 CSFs to 2012/13 BASELINE data (Fig 1). Ninety-five % confidence intervals are provided for the slope and intercept values.

<b>Α</b> β <sub>1-42</sub>	t-tau	p-tau <sub>181</sub>
Y = 0.99X + 1.45	Y = 1.008X - 0.89	Y = 1.04X - 1.11
1.45(-8.9 - 9.4)	-0.89(-4.8 - 3.8)	-1.11(-4.5 - 1.74)
0.992(0.94 - 1.06)	1.008(0.95 - 1.07)	1.04(0.95 - 1.14)
0.905	0.902	0.81
	$A\beta_{1-42}$ $Y = 0.99X + 1.45$ $1.45(-8.9 - 9.4)$ $0.992(0.94 - 1.06)$ $0.905$	$A\beta_{1.42}$ t-tau $Y = 0.99X + 1.45$ $Y = 1.008X - 0.89$ $1.45(-8.9 - 9.4)$ $-0.89(-4.8 - 3.8)$ $0.992(0.94 - 1.06)$ $1.008(0.95 - 1.07)$ $0.905$ $0.902$

**Figure 2.** Passing-Bablok linear regression analysis plots of for comparing RAW CSF concentration results obtained in 2015 with those obtained in the analyses performed in 2013(referred to as "2013T") transformed to 2007 BASELINE as described in "*ADNI GO and ADNI 2 First batch analyses of CSF biomarkers: revised anchoring to 2007 ADNI 1 BASELINE dataset procedure"* ). The linear regression equations derived from these analyses for each of the 3 biomarkers were used to rescale or Transform the RAW 2015 dataset to the 2013 (UPENNBIOMK6) data sets BASELINE data that had been rescaled or Transformed to the 2007 BASELINE dataset (see Figure 1 for the comparisons, by linear regression analyses, of the A $\beta_{1-42}$ , t-tau and p-tau<sub>181</sub>2015T data with the 2013T data).

<u> Aβ<sub>1-42</sub>:</u>





## <u>t-tau:</u>



<u>p-tau<sub>181</sub>:</u>





#### References

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