An Overview of the first 8 ADNI CSF Batch Analyses

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EXECUTIVE SUMMARY

This Methods report provides an overview for the series of 8 datasets reported by the ADNI Biomarker Core from 2008 through 2015 on the ADNI/LONI website. Accompanying this report is a .CSV datafile, "All UPENNBIOMKs" master dataset in which each of the 8 previously reported data sets are combined to permit easier access to all of the CSF $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} concentration data generated using the Research Use Only (RUO) INNO-BIA AlzBio3 immunoassay (Fujirebio, Belgium). In this review we provide routine quality control performance data for the ADNIGO/2 datasets, describe the re-scaling procedure used for the datasets that followed the original ADNI1 BASELINE dataset and the rationale for this re-scaling procedure, as well as discuss comparisons between re-scaled to raw data and the limitations of this procedure. In the Conclusions section we provide some recommendations for data analyses. It is our hope that these data descriptions and discussions will further facilitate the use of the ADNI CSF $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} datasets for other biomarker investigators.

BACKGROUND

From the inception of the ADNI1 study, and throughout the ADNIGO and ADNI2 phases of the study, all CSF $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} concentration measurements have been made using the micro-bead-based multiplex immunoassay, the INNO-BIA AlzBio3 RUO test (Fujirebio, Ghent, Belgium)(1), on the Luminex platform.

Prior to implementation of this test system in the ADNI1 study, extensive within- and between-laboratory validation studies were conducted using non-ADNI CSF samples(2,3). Upon completion of these validation studies the first batch analysis results for 410 ADNI1 BASELINE CSF sample aliquots was reported on the ADNI website and subsequently described in the peer-reviewed literature(2). These studies confirmed earlier documentation of the very good (<10% run-to-run with a single lot of reagents and calibrators)(1) within-laboratory precision but more variable center-to-center reproducibility (inter-center %CV 95% CI range of 15.9–19.8% (mean=17.9%) for $A\beta_{1\text{–}42}$, 9.6–15.2% (13.1%) for t-tau and 11.3–18.2% (14.6%) for p-tau_{181} (2,3).

A limitation of RUO immunoassay tests such as the AlzBio3 immunoassay is kit lot to kit lot variation in measured concentrations. In the absence of an international reference material against which to standardize each new lot of reagents and calibrators we have chosen in the ADNI1/GO/2 phases to use the original BASELINE dataset (UPENNBIOMK) as the "gold standard" against which to re-scale or anchor the subsequent batch ADNI CSF sample runs. An important basis for considering this dataset as the "gold standard" set is that the same kit and calibrator lot used for the original ADNI BASELINE analyses was used for a set of ADNI-independent CSFs (provided by the UPENN NIA funded Alzheimer's Disease Core Center or ADCC) from autopsy-confirmed AD subjects and age-matched living controls. The latter provided for determination of cutpoint concentration values for CSF $A\beta_{1\text{-}42}$, t-tau

and p-tau$_{181}$ that were then applied to the ADNI BASELINE dataset as previously described(2) and have been used as reference values for ADNI1,GO and 2 studies(2, 4-10).

The requirement to re-test pristine BASELINE aliquots from longitudinal sample sets in the subsequent batches has provided the resource needed-the replicate BASELINE CSF pristine aliquots, for the re-scaling process used in BIOMK's 2-8. It should be noted here that due to the concern about overuse of BASELINE ADNI1 CSF pristine aliquot samples we undertook a transition to the use of ADNIGO/2 BASELINE aliquot samples that were rescaled to ADNI1 BASELINE during analyses of the UPENNBIOMKs 5 and 6 CSF samples as described in section 5 below.

Integral to the ADNI study is measurement, as reliably as possible, of longitudinal changes in all biomarkers that are measured over the course of the study. Thus the batch analyses, except for #5, following the ADNI1 BASELINE dataset included sets of two or more longitudinal ADNI subject CSF samples in order to measure as reliably as possible within-subject changes in Aβ_{1-42}, t-tau and p-tau$_{181}$ concentrations. As the number of within-subject longitudinal samples accrued, updated longitudinal CSF samples were analyzed in subsequent batch runs and included analyses of pristine replicate BASELINE CSF aliquots from ADNI1 subjects.

Reliable measurement of longitudinal changes in CSF AD biomarkers is challenging since the annual changes involved are frequently smaller than the 5-10% variability obtained in highly quality-controlled within-laboratory test performance using a single lot of reagents (Table 1 and 11-14). The lot-to-lot variability, coupled with run-to-run variance, can be such that total variance is greater than 10% for replicate analyses of aliquots prepared from the same original CSF sample but measured one or more years apart (15) (see Figure App1A,B,C in the Appendix for replicate analyses of BASELINE CSF aliquots from ADNI subjects comparing the %CV for replicate samples of raw data to that obtained using the raw data re-scaled to the original BASELINE results for replicate samples). The re-scaling process, by overcoming lot-to-lot variability, thereby provides improved precision performance across time and across different batches of reagents. By normalizing subsequent batch analyses to the original ADNI1 BASELINE data this re-scaling process enables the use of the ADNI1 cutpoints for clinical utility studies of ADNIGO and ADNI2 CSF data. In the subsequent batch runs individual subject longitudinal sample sets were included in order to update the longitudinal profiles data. This was done to meet the commitment made by ADNI leadership to provide timely reports of these data and for use by the scientific community for analyses.

Following the initial batch run are 7 subsequent batch runs, including ADNI1, ADNI GO and ADNI2 subject CSFs. The batch runs in UPENNBIOMKs 2-4 included updated longitudinal sample sets. The second through the fourth batch analyses (UPENNBIOMKs 6-8) run during the ADNIGO and ADNI2 phases included BASELINE and follow-up longitudinal samples (see App Table 1 in Appendix). Each batch run utilized a different lot number of AlzBio3 immunoassay kits and calibrators (the lot numbers for each batch are listed in the accompanying .CSV file, referred to as ***" All UPENNBIOMKs" master data set***).

REVIEW OF THE 8 ANALYSIS REPORTS FOR ADNI SUBJECT CSF SAMPLES

Routine Quality Control. In each of the batch runs pristine aliquots of two CSF pools were included for the purpose of monitoring the performance of each daily analytical run. In addition, the performance of these pools over the time period of the batch reflects run to run precision. A summary of performance of analyses of these QC samples is summarized for

the ADNI1 study in two publications (2,3). The sources for the CSF pools used in ADNI1 were non-ADNI residual samples. As part of routine quality control for the analysis of the ADNIGO and ADNI2, CSF samples CSF pools (#53 and #54) were created from residual ADNI1 CSF left over samples following analysis. These two CSF pools were used in the 4 batch analyses in the ADNIGO/2 phases and the precision performance is summarized in Table 1 below. The within-batch precision values were <10% for $A\beta_{1-42}$, t-tau and p-tau_{181}(5.1-7.8%, 4.4-9.8% and 5.1-8.8%, respectively). These data also show a good level of assay stability over the time period from 2/2012 through 2/2015 (overall mean value ranges were: 136-150 and 222-254 pg/mL, $A\beta_{1-42}$; 113-132 and 59-70 pg/mL, t-tau; 25.4-28.9 and 19.1-21.3 pg/mL, p-tau_{181}) (see summary in Table 1).

Table 1. Summary QC statistics for ADNIGO/2: CSF pools #53 and #54, Feb 2012 through Feb 2015.

YEAR	2012	2013	2014	2015	2012	2013	2014	2015
	ADNI CSF pool #53				ADNI CSF pool#54			
N	13	25	16	13	12	25	16	10
$A\beta_{1-42}$	136±6.9	148±9.4	150±8.8	140±8.6	222±15	236±18	254±15	240±17
%CV	5.1%	6.3%	5.9%	6.1%	6.7%	7.8%	6.0%	7.2%
t-tau	132±8.4	128±10	118±8.0	113±6.5	70.3±3.1	65.9±6.4	59.3±5.4	61.1±3.3
%CV	6.4%	8.1%	6.7%	5.7%	4.4%	9.8%	9.1%	5.4%
P-tau_{181}	25.4±1.4	26.5±1.4	28.9±2.5	27.5±1.5	19.1±0.9	19.1±1.2	20.4±1.2	21.3±1.6
%CV	5.6%	5.1%	8.8%	5.3%	5.1%	6.1%	5.7%	7.4%
Kit lot#	220093	225445	237092	242298	220093	225445	237092	242298
Stds lot#	215382	225736	236947	236947	215382	225736	236947	236947

The 8 datasets. The following is a brief review describing essential features of the datasets generated for each of the 8 reports. This is followed by a comparison of the re-scaled data to raw data including comparison of precision of the replicate $A\beta_{1-42}$, t-tau and p-tau_{181} values, concordance of CSF $A\beta_{1-42}$ values(re-scaled and raw) with amyloid-β Florbetapir PET scan SUVR values, a comparison of re-scaled vs raw longitudinal profiles and predictive performance, as assessed by comparing above and below cutpoint values, of $A\beta_{1-42}$ alone and the t-tau/$A\beta_{1-42}$ ratio, for cognitive and functional decline using ADNIGO/2 re-scaled data and the cutpoints established in ADNI1, and prediction of progression to dementia for ADNIGO/2 mild cognitive impairment (MCI) subjects.

At the conclusion of this report, we provide basic recommendations for investigators who may be using these CSF biomarker data for the first time to explore questions such as relationships between BASELINE data and imaging biomarkers, and to clinical events over time or, characterization of the longitudinal trajectories of the CSF biomarkers in relationship to imaging measurements and to clinical parameters. In order to facilitate efficient use of

the data from the 8 reports of the batch runs we have combined the re-scaled and raw data into one master data set in .CSV file format, referred to as ***" All UPENNBIOMKs".***

First Batch.

1. The first batch analysis of ADNI1 BASELINE CSF sample aliquots was completed in late 2007 and reported in early 2008. The $A\beta_{1-42}$, t-tau and p-tau_{181} concentration data for 410 BASELINE CSFs were originally reported in the .csv file UPENNBIOMK. Using the same lot of reagents and calibrators these biomarkers were also measured in an ADNI-independent set of pre-mortem CSF samples from 56 autopsy-confirmed AD cases and 52 cognitively normal living elderly subjects provided by the UPENN ADCC (2). The ADNI1 BASELINE data serve as the reference set for the ADNI study and replicate BASELINE aliquots have served as reference samples for subsequent batches of CSF analyses. This has been necessary since there is inherent immunoassay kit lot to lot variance in calibration in the RUO AlzBio3 method and there has not been available Certified Reference Material (CRM) in human CSF to permit checking the calibration of each new lot of kit standards prior to their use. Since CSF samples are an invaluable and irreplaceable resource the use of the extra aliquots of BASELINE CSF samples was discussed with the ADNI Executive Committee and was as conservative as possible and based on the requisite inclusion of original BASELINE aliquots in subsequent runs of longitudinal sample sets.

Second Batch.

2. The second batch analysis of ADNI1 CSF included 328 year 1(12 month collection) CSF samples and the corresponding matched fresh BASELINE aliquots in addition to remaining BASELINE-only aliquots for a total of 410 BASELINE and 328 year 1 aliquots obtained in ADNI1. This dataset is included in the .csv file UPENNBIOMK2, uploaded in the first quarter of 2009. We included each longitudinal pair of aliquots on the same 96 well plate in order to control for run to run variance (%CV of approximately 5-10%) which is higher than the annual rate of change in biomarker concentration in many cases(11-14). In this second batch analysis fresh 410 BASELINE results were compared to the corresponding values in the original BASELINE dataset in UPENNBIOMK and detected a high bias in the UPENNBIOMK2 $A\beta_{1-42}$ results compared to the original data (see Figure 1A below). Little evidence for bias was observed in t-tau between the original data and that obtained in the second batch run(Figure 1B), whereas the variance for p-tau_{181} between the two batch runs was very high for a subset of samples (Figure 1C). We attribute the high bias for $A\beta_{1-42}$ concentration to reagent and calibrator lot to lot variation, and according to the manufacturer (Fujirebio Europe) was likely due to a change in a buffer constituent. We believe that the batch to batch variance for a subset of p-tau_{181} samples reflects a matrix effect but further work would have to be done to better define this. Another possibility, sample mis-identification is unlikely given the lack of this disparity for t-tau (Figure 1B) and the fact that subject CSF sample and reaction mixture is always the same for each CSF sample for $A\beta_{1-42}$, t-tau and p-tau_{181} in this multiplexed immunoassay. Over the span of time of these batch analyses there has been no change in the monoclonal capture or detection antibodies or the method of production of the bead based reagents or any other constituent of the AlzBio3 RUO immunoassay kits.

Re-scaling procedure. Since the original ADNI1 BASELINE set is the reference set of sample aliquots for comparison, a linear regression-based re-scaling (anchoring) procedure was put into place at that time in conjunction with the second batch dataset for $A\beta_{1-42}$ and t-tau (Figure 1D, 1E and 1F). The procedure makes use of fresh ADNI BASELINE CSF aliquots within each of the subsequent batch runs. The linear-regression based re-scaling process uses the slope and Y-intercept values obtained for

$A\beta_{1-42}$, t-tau and p-tau181 (Figure 1A,1B,1C, respectively) and solves the regression equation for X_i where X_i are the rescaled values for each RAW result, $Y_{i.}$ Thus, the equation Xi = Yi-(-21.9)/1.3 is the equation used to generate re-scaled values for CSF $A\beta_{1-42}$ for the second batch results. The re-scaled values X_i are obtained by solving this equation for each Y_i raw result. To demonstrate the improved agreement between these re-scaled data and the original BASELINE values, linear regression plots of re-scaled UPENNBIOMK2 vs original UPENNBIOMK data are shown in Figure 1D 1E and 1F for $A\beta_{1-42}$, t-tau and p-tau_{181}, respectively. Each of the BASELINE and YR1 raw $A\beta_{1-42}$, t-tau and p-tau181 results were thus re-scaled using the above linear equation specific for each biomarker. In subsequent batch analyses we used the same linear regression method for re-scaling the batch-specific raw $A\beta_{1-42}$, t-tau and also p-tau_{181} to their BASELINE results. The r^2 values for $A\beta_{1-42}$ and t-tau of 0.89 and 0.95 respectively are consistent with reasonably good correlation between 2008 raw and the original baseline data, and for re-scaled vs original baseline data for $A\beta_{1-42}$ and t-tau. However, the r^2 value of 0.66 for p-tau_{181} indicates to us some type of heterogeneity for a small subset of p-tau_{181} CSF samples and this limits the value of the p-tau data both re-scaled or raw. Further investigation will be required to better understand this heterogeneity for p-tau_{181} and correct it and to determine a diagnostically useful cutpoint for ADNIGO/2 subjects.

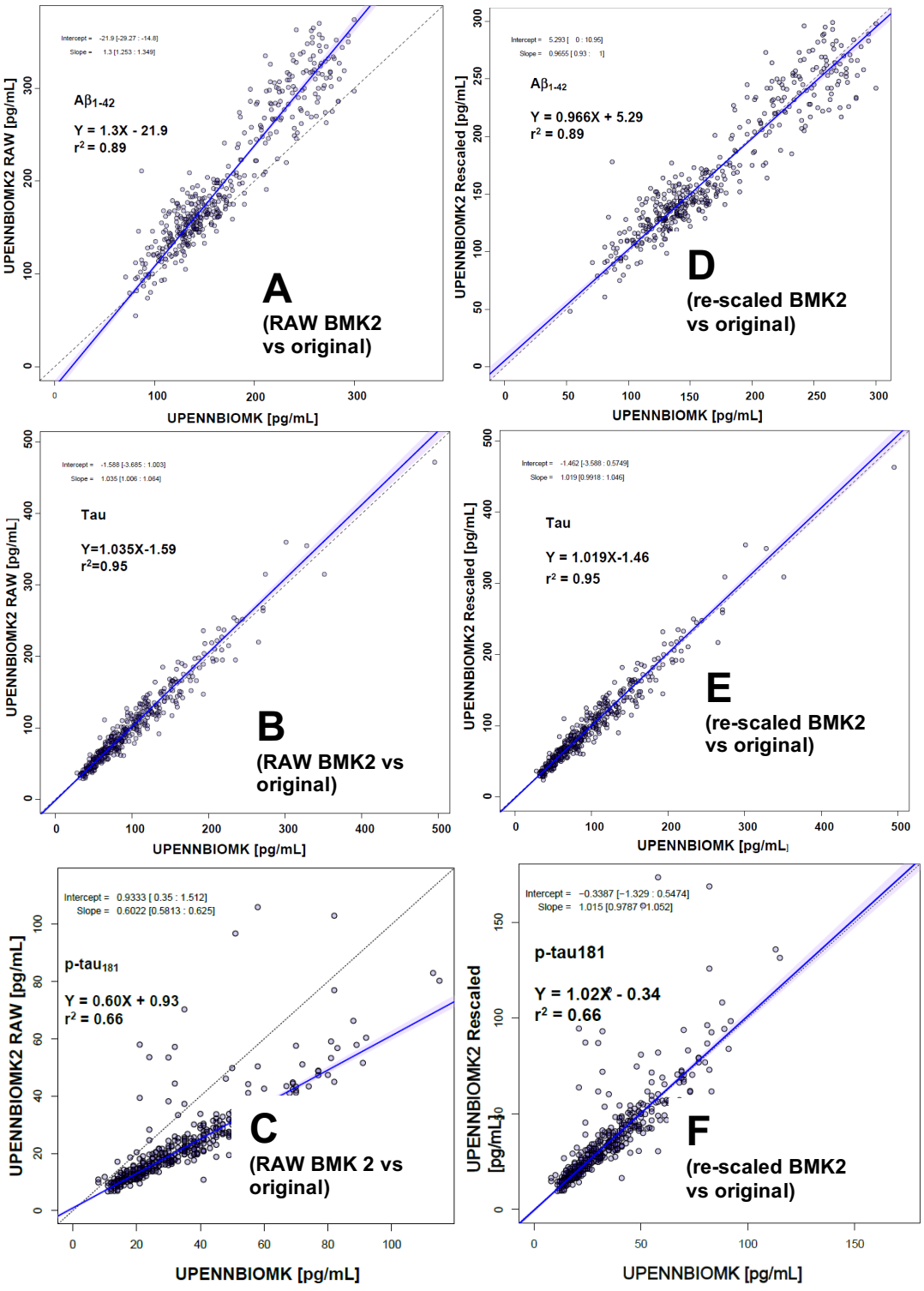


Figure 1. A,B&C. Deming linear regression plots of RAW $A\beta_{1-42}$,t-tau or p-tau_{181}data from UPENNBIOMK2(2008) BASELINE CSF analyses vs the original BASELINE data from UPENNBIOMK(2007). D,E, F. Deming plots of re-scaled 2008 datasets vs 2007 original BASELINE.

Since each subsequent lot of immunoassay reagents and calibrators has inherent bias, the re-assayed BASELINE samples in that batch served for the re-scaling for that batch. The overall impact of re-scaling the raw data in the subsequent 7 datasets on precision of replicate analyses(re-scaled vs raw replicates over the years) is summarized in Appendix Figures App1A, App1B and App1C. This comparison shows between-batch mean %CV values of 7.3%(re-scaled)

vs 22.1%(raw) for $A\beta_{1-42}$, 10.2%(re-scaled) vs 35.4%(raw) for t-tau and 14.4%(re-scaled) vs 18.5%(raw) for p-tau_{181}. Furthermore, these data show that the improved precision holds for non-BASELINE longitudinal CSF Aβ1-42 to an equivalent degree as compared to the impact on BASELINE-only replicate samples (%CV range of 6.5-8.3% BASELINE replicates, 6.6-8.1% Yr1, 7.1% Yr2 and 8.3% Yr3 replicates) supporting the lack of bias in the re-scaling process for longitudinal samples(Appendix AppTable 1).

Third Batch.

3. 91 sets of "triplet" CSF samples were included in the mid-2009 batch run, reported in UPENNBIOMK3, the first report of the add-on ADNI longitudinal CSF biomarker study (BASELINE, YR1 and 24 and/or 36 month CSFs) funded by an anonymous donor. Linear regression-based anchoring as described above for the second batch, but using the 91 BASELINE ADNI1 fresh aliquots analyzed in this third batch, was used for rescaling the third batch run dataset to the original BASELINE dataset. This dataset was uploaded in the 4th quarter of 2009. The results in batch UPENNBIOMK3 were obtained using an alternative experimental protocol that was devised to solve a recurring performance problem of the assay, experienced in the Biomarker Core, low bead counts for the t-tau measurement. This assay problem was fixed by the manufacturer in the subsequent reagent lots, and therefore the alternative protocol was no longer needed and no longer used for subsequent analyses. All results in this batch were replaced, and expanded upon by additional later longitudinal samples by results in UPENNBIOMK4.

Fourth Batch.

4. Test results for 142 ADNI1 subjects with 3-5 visits each are reported in UPENNBIOMK4, uploaded in the 3rd quarter of 2012. This dataset includes CSF $A\beta_{1-42}$, t-tau and p-tau_{181} BASELINE plus longitudinal results for 142 subjects for a total of 495 CSF samples, analyzed over a two year period, early 2009 through early 2011. During the time period of the third and fourth batch analyses we changed from use of Deming linear regression to Passing-Bablok since the latter performs better with the smaller numbers of replicate BASELINE samples involved in these studies. Over this(UPENNBIOMK4) two year period of time 5 different lots of immunoassay reagents were used. Bridging to ADNI1 BASELINE was done as described above for each of these batches that comprise this fourth set of results. Graphical displays of these analyses are provided in App Figure 2. The results in this set of analyses replace and expand upon those initially reported in UPENNBIOMK3 as noted above.

Fifth Batch.

5. Initial test results for 390 BASELINE ADNIGO + ADNI2 subjects are reported in UPENNBIOMK5. There were no longitudinal sets included in this batch run. The initial procedure anchoring these 2012 raw concentration data to 2007 ADNI 1 BASELINE CSF $A\beta_{1-42}$, t-tau and p-tau_{181} concentrations was based on the use of a limited number of never before thawed 2007 BASELINE CSF aliquots. Thus, a total of twelve (12) 2007 aliquots were selected for inclusion in this fifth batch run based on two criteria: 1)at least 20 aliquots were available for each selected patient CSF; 2) selection of six sets of two aliquots was based on ascending $A\beta_{1-42}$ concentrations over the range observed for the 2007 BASELINE samples. We included these 12 CSF aliquot samples in one analytical run performed in early 2012, using the same lot of AlzBio3 reagents as used for the ADNI CSFs run in 2012

and used the Passing-Bablok (P-B) regression line to anchor the data set to 2007. At the time we did these analyses we assumed that the relationship between the 2012 and 2007 studies captured by analysis of these 12 samples in one analytical run would be representative of the whole set of 2007 ADNI 1 BASELINE samples. However, as shown in Appendix Figure App 3, this assumption was not correct, having under-transformed all 3 biomarkers. There was no precedent upon which to base our initial selection of the number of replicates and runs to use in these studies. Hence, the findings shown in Appendix Figure App3 were unexpected, and, in light of the current data, however, we conclude that to capture the natural variance in $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} concentration measurements and achieve accurate anchoring to the 2007 ADNI 1 BASELINE set a larger number of CSF samples and runs are needed. From our ongoing experience, at least 20 CSF samples analyzed over at least 3 runs is required.

The revised strategy for anchoring the 2012 CSF BASELINE dataset (n=390) that we believe provided a reasonable approach to re-scaling to the 2007 ADNI1 BASELINE CSF results is to use the P-B regression equation, produced by the re-scaling of the 2013 ADNI CSF data set, for re-scaling the 2012 dataset. We believe this is a sound approach since the lot-to-lot performance was unusually close between 2012 and 2013 (Table 1). The regression analysis results for the 2012 CSF biomarker re-scaled data using this approach ("UPENNBIOMK5 re-scaled"") shown in Appendix Figure App4C confirm the success of this re-scaling procedure. This regression analysis compared the 25 BASELINE samples assayed in 2012 (and re-scaled according to the described method that used the PB regression parameters from 2013 re-scaling to ADNI1 BL) to replicate aliquots of those 25 BASELINE ADNIGO/2 samples that were included in the 2013 run(and re-scaled to ADNI1 original BL).

Sixth Batch.

6. The batch analyses reported in UPENNBIOMK6, uploaded 2nd Quarter, 2013, included 692 CSF aliquot samples(368 were BASELINE CSF samples from ADNIGO+2 subjects and 324 were ADNI1+2 longitudinal subject CSF samples). The latter included 62 pristine 2007 ADNI1 BASELINE CSF samples that served for re-scaling of the RAW results from this batch of analyses to the 2007 ADNI1 BASELINE results.

Seventh Batch.

7. The UPENNBIOMK7 batch analyses, uploaded 3rd Quarter, 2014, included 275 BASELINE plus 153 year 2 or 2.5 CSF aliquot samples from ADNIGO/2 study subjects. The RAW CSF results for the 147 ADNI2 BASELINE samples included in this run, that had been run in either 2012 or 2013 and re-scaled according to 5 and 6 above to 2007 BASELINE, were re-scaled to those results.

Eighth Batch.

8. Batch analysis results for UPENNBIOMK8 were uploaded in the 2nd Quarter of 2015 and included 230 ADNI 2 CSF samples (112 pairs of BASELINE + 24 months; five (5) 24-month-only and 1 BASELINE only). Raw CSF data for the 113 BASELINE aliquot samples were re-scaled to the 2013 re-scaled data as described above.

OVERVIEW OF CSF $A\beta_{1-42}$, t-tau and p-tau_{181} ACROSS THE ADNIGO/2 COHORTS

General statistics. Table 2 below summarizes the general CSF biomarker statistics for ADNIGO/2 subjects by clinical diagnosis.

Table 2. Cerebrospinal fluid AD biomarker concentrations, t-tau/$A\beta_{1-42}$ ratio and riskTAA2i logistic regression model values in ADNIGO+2 subjects at BASELINE. All ADNIGO+2 subjects who provided BASELINE CSF, first time tested, are included in this summary table.

	$A\beta_{1-42}$ (pg/mL)	t-tau (pg/mL)	p-tau_{181} (pg/mL)	t-tau/$A\beta_{1-42}$	riskTAA2i
AD (n=132)					
Median	131	115	53	0.92	0.92
Mean±SD	137±37	132±65	59±34	1.04±0.60	0.80±0.27
LMCI (n=155)					
Median	144	88	42	0.67	0.76
Mean±SD	159±50	101±56	48±27	0.72±0.48	0.62±0.38
EMCI (n=276)					
Median	185	63	32	0.33	0.26
Mean±SD	184±51	76±48	37±21	0.49±0.46	0.39±0.36
SMC (n=96)					
Median	207	58	31	0.27	0.14
Mean±SD	201±49	65±31	38±21	0.36±0.23	0.30±0.32
NC (n=161)					
Median	204	57	30	0.28	0.15
Mean±SD	195±51	68±33	34±19	0.39±0.27	0.33±0.33

Mann-Whitney test: $p<0.0001$ for each of the five biomarker tests for AD vs NC, AD vs SMC and AD vs EMCI; for AD vs LMCI: $p<0.0001$ for $A\beta_{1-42}$, t-tau and tau/$A\beta_{1-42}$; $p<0.001$ for riskTAA2i; $p<0.01$ for p-tau_{181}; p<0.0001 for NC vs LMCI for each of the five biomarker tests; for NC vs EMCI: $p=0.032$, 0.27, 0.48, 0.19 and 0.0984, respectively, for $A\beta_{1-42}$, t-tau, p-tau_{181}, t-tau/$A\beta_{1-42}$ and riskTAA2i ; for LMCI vs EMCI: $p<0.0001$ for each of the five biomarker tests. These data are consistent with increased AD neuropathology, based on each of the five CSF biomarker tests, as we move from NC or SMC to EMCI, then to LMCI and ultimately to AD. riskTAA2i is a logistic regression model that includes Aβ1-42, t-tau and *APOE* ε4 allele # AGE and GENDER: riskTAA2i=1/(1+exp(expression)), where expression = 9.6304 – APOE + Gender + 0.00730*AGE + 0.03266*ABETA – 0.02126*TAU, where: APOE = 11.5724(2/4) or 13.4592(2/3) or 14.1990(3/3) or 14.5694 (3/4) or 31.9817(4/4) and GENDER = 0(sex=F) or 0.6632(sex=M).

Table 3. Mean±SD values for CSF biomarkers for ADNIGO+2 subjects by diagnosis and stratified by *APOE* ε4 allele number. These BASELINE ADNIGO/2 data are the first BASELINE CSF $A\beta_{1-42}$, t-tau and p-tau_{181} data to have been measured in an individual and who had APOE e4 genotyping data available. The CSF biomarker concentration data are from the ADNIGO/2 datasets from the "All UPENNBIOMKs" master datafile.

***APOE* ε4 allele#**		**$A\beta_{1-42}$**	**t-tau**	**p-tau_{181}**	**t-tau/$A\beta_{1-42}$**	**riskTAA2i**
AD		**mean±SD**				
0	(n=44)	155±49	121±59	52±33	0.85±0.46	0.63±0.36
1	(n=62)	133±25	136±68	61±35	1.1±0.59	0.85±0.17
2	(n=26)	115±26	143±66	66±33	1.33 ±0.74	1.00±0.001
MCI						
0	(n=66)	190±50	77±53	37±21	0.46±0.39	0.35±0.33
1	(n=63)	141±35	122±53	58±31	0.92±0.48	0.78±0.27
2	(n=26)	121±29	114±46	53±23	0.96±0.34	1.00±0.001
EMCI						
0	(n=154)	201±48	63±33	31±17	0.35±0.27	0.26±0.29
1	(n=92)	169±47	87±49	42±23	0.60±0.47	0.52±0.34
2	(n=16)	124±22	137±85	56±27	1.22	1.00±0.001
SMC						
0	(n=63)	216±42	59±27	32±17	0.29±0.16	0.18±0.22
1	(n=32)	171±47	76±37	49±24	0.49±0.30	0.52±0.34
NC						
0	(n=117)	203±47	66±34	32±16	0.37±0.27	0.25±0.29
1	(n=38)	183±54	74±34	40±25	0.45±0.29	0.46±0.33
2	(n=6)	116±31	57±20	41±19	0.52±0.24	1.00±0.001

p: e4 1 vs 0, <0.0001 for $A\beta_{1-42}$ for each of the above 5 clinical cohorts; t-tau: <0.0001 for EMCI & MCI,<0.01 for NC and SMC, 0.581 for AD; p-tau_{181}:<0.0001 for NC, EMCI & MCI, <0.01 for SMC, 0.384 for AD; t-tau/$A\beta_{1-42}$: <0.0001 for NC, EMCI& MCI, <0.001 for SMC, 0.063 for AD; riskTAA2i:<0.0001 for NC, SMC, EMCI & MCI, 0.022 for AD.

These data show, as expected, a significant decline in $A\beta_{1-42}$ concentration with increased *APOE* ε4 allele # with a comparable degree of decline as reported for the ADNI1 subjects(2) in NC, LMCI and AD. As in Table 2, the first occurring BASELINE data from the "All UPENNBIOMKs" master dataset .csv file were used for these calculations for all ADNIGO/2 subjects who had *APOE* genotyping data.

APPLICATION OF THE CSF $A\beta_{1-42}$, t-tau/$A\beta_{1-42}$ CUTPOINTS FROM ADNI1 TO ADNIGO/2.

A. Mixture model-based cutpoint for $A\beta_{1-42}$ in ADNIGO/2 subjects. The cutpoint value in re-scaled ADNIGO/2 subjects was assessed using the unbiased mixture modeling statistical method taking advantage of the bimodal distribution observed for $A\beta_{1-42}$ (2,4)(Fig2 below). The cutpoint value for $A\beta_{1-42}$ from the mixture modeling analysis of the 239

ADNIGO/2 AD and NC subjects who had at least one Florbetapir amyloid-β plaque PET scan is 187 pg/mL, a value consistent with the value of 192 pg/mL obtained for ADNI1. For reference purposes the corresponding cutpoint value using the raw data is 254 pg/mL. This value is in good agreement with a cutpoint value established in an ADNI-independent pharmaceutical trial, the Solanezumab Expedition 3 trial, which established a cutpoint value of 249 pg/mL using the INNO-BIA AlzBio3 and CSF data from the Solanezumab Expedition 1 and 2 trials (17).

B. Concordance between CSF $A\beta_{1\text{-}42}$ and Florbetapir Amyloid-β PET scan SUVR values. Analyses of the concordance between CSF $A\beta_{1\text{-}42}$ and Amyloid-β PET scan SUVR values are shown in Figure 2 below. These analyses show ≥90% concordance for the early AD and late MCI ADNIGO/2 subgroups(94.2 and 90.3%, respectively) and decreasing concordance values for early MCI (EMCI)and cognitively normal subjects (86.3 & 82.4%, respectively). These results provide confirmation for the observations of others for generally very good concordance between these two measures of amyloid plaque burden but with increased differences moving from late MCI to earlier stages of AD pathology (8,9,16). Further studies including longer observation times will be required to deepen our understanding of the reasons for the increased differences at earlier time points in the disease course trajectory, and is beyond the scope of this ADNI Methods report. These data provide further support for the validity of the re-scaling process used for the ADNIGO/2 subject CSFs for $A\beta_{1\text{-}42}$.

No bias produced by "anchoring"on interpretation of CSF $A\beta_{1\text{-}42}$

ADNIGO + 2, ***anchored to 2007***;
CSF $A\beta_{1\text{-}42}$ concordance with FBP using Mixture Modeling (MM) cutpt

	concord	discord	FBP-/Aβ-	FBP+/Aβ+	FBP-/Aβ+	FBP+/Aβ-	*concordance*
NC n=153	126	27	85	41	23	4	0.824
EMCI n=256	221	35	119	102	23	12	0.863
LMCI n=144	130	14	35	95	11	3	0.903
AD n=86	81	5	4	77	5	0	0.942
All n=639	558	81	243	315	62	19	0.873

●●ADNI subjects are all ADNIGO+2 AD+NC who had both lp & FBP●●

ADNIGO+2, ***RAW data***;
CSF $A\beta_{1\text{-}42}$ concordance with FBP using MM cutpt

	concord	discord	FBP-/Aβ-	FBP+/Aβ+	FBP-/Aβ+	FBP+/Aβ-	*concordance*
NC n=153	126	27	85	41	23	4	0.824
EMCI n=256	221	35	119	102	23	12	0.863
LMCI n=144	130	14	35	95	11	3	0.903
AD n=86	81	5	4	77	5	0	0.942
All n=639	558	81	243	315	62	19	0.873

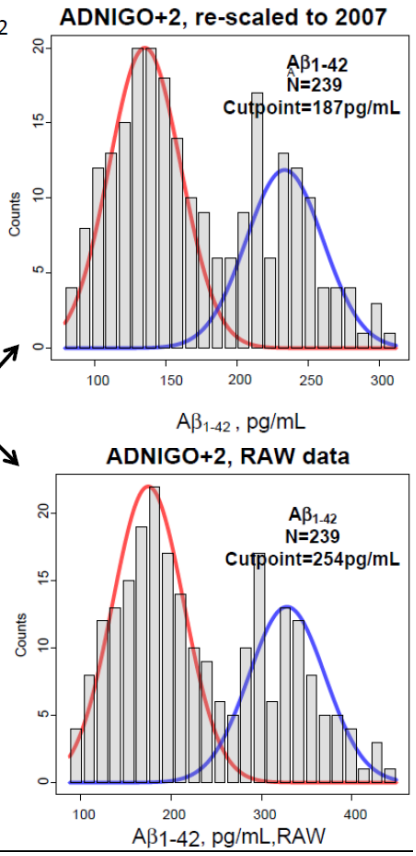


FIGURE 2. CSF $A\beta_{1\text{-}42}$ concordance with Florbetapir PET Amyloid-β plaque burden SUVR data for ADNIGO/2 subjects who had an lp within ± 90 days of PET scan. The SUVR cutpoint value of 1.11, recommended for cross-sectional studies(12) by Landau and Jagust, and the CSF $A\beta_{1\text{-}42}$ value of 187 pg/mL defined for ADNIGO/2 study subjects were used here for these concordance assessments. For comparison purposes we include in this figure the concordance results obtained when raw data for BASELINE CSF $A\beta_{1\text{-}42}$ is used.

C. *Prediction performance for cognitive and functional decline*

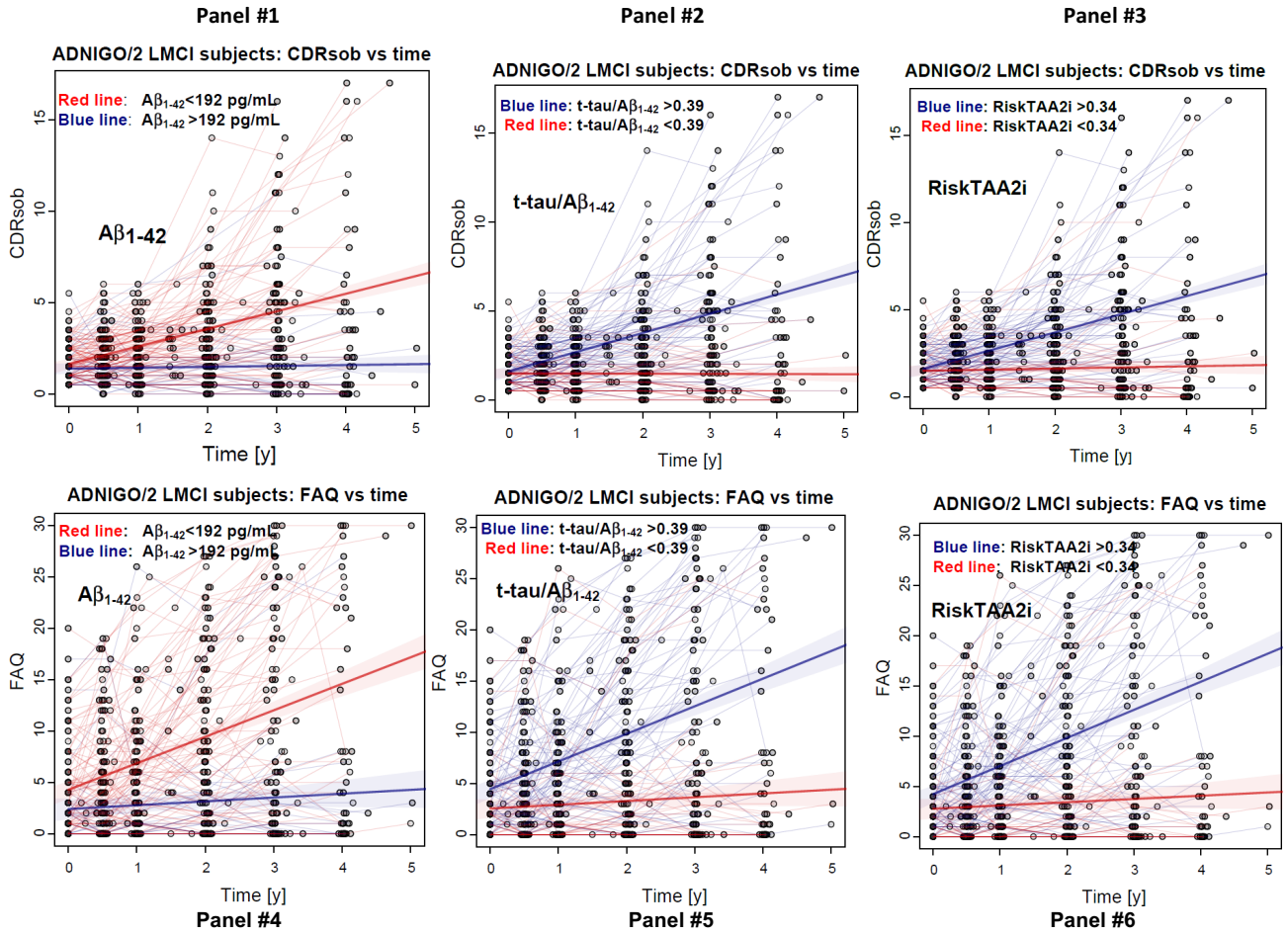


Figure 3. Panels 1-3, Spaghetti plots, in ADNIGO+2 subjects of CDR sum of boxes, or FAQ(panels 4-6), vs time(y), with superimposed linear regression lines for CDRsob values associated with (panel 1)$A\beta_{1\text{-}42}$ values above (blue line) or below (red line) the 192 pg/mL cutpoint value; panel 2, t-tau/$A\beta_{1\text{-}42}$ values above (blue line) or below (red line) the cutpoint value of 0.39; panel 3, logistic regression model (includes $A\beta_{1\text{-}42}$, t-tau and *APOE* ε4 allele # as covariates(2)) values above(blue line) & below(red line) the cutpoint value of 0.34.

Table 4. Annual change for CDRsob & FAQ in subjects with pathologic or non-pathologic BASELINE CSF (first available), defined by cutpoints for $A\beta_{1\text{-}42}$, t-tau & RiskTAA2i in ADNIGO/2 LMCI subjects.

Cutpoint	**192 pg/mL**	**0.39**	**0.34**
CSF Biomarker	$A\beta_{1\text{-}42}$	t-tau/$A\beta_{1\text{-}42}$	RiskTAA2i
CDRsob			
CSF pathologic	0.90±1.1/year	1.00±1.1/year	0.94±1.1/year
CSF non-pathologic	0.016±0.64/year	0.0098±0.62/year	0.08±0.71/year
p	<0.0001	<0.0001	<0.0001
FAQ			
CSF pathologic	2.62±2.96/year	2.82±2.93/year	2.79±2.91/year
CSF non-pathologic	0.25±0.92/year	0.34±1.41/year	0.29±1.43/year
P for CSF pathologic vs non-pathologic	<0.0001	<0.0001	<0.0001

These data confirm the earlier observations in ADNI1 subjects of significant predictive performance for Aβ1-42 and t-tau/Aβ1-42 below and above their respective cutpoint values for cognitive decline or decline in functions of daily living (5.6).

D. *Prediction of progression to AD dementia in ADNIGO+2 MCI subjects*

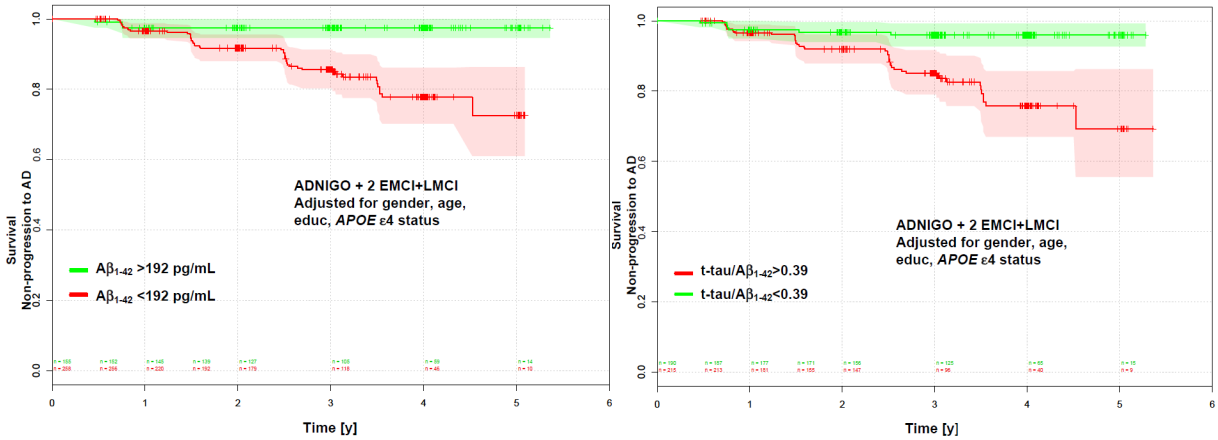


Figure 4. Kaplan-Meyer survival plots for ADNIGO + ADNI2 EMCI+LMCI subjects. Cox-proportional Hazards models adjusted for gender, age, education and *APOE* ε4 status were developed for CSF Aβ1-42 alone and for t-tau/Aβ1-42 ratio for above and below the respective cutpoint values of 192 pg/mL and the ratio value of 0.39. The shaded areas around each survival curve are the 95% confidence intervals.

CONCLUSIONS AND RECOMMENDATIONS

This review of ADNI CSF methods provides routine quality control performance data for the ADNIGO/2 datasets, describes the re-scaling procedure used for the datasets that followed the original ADNI1 BASELINE dataset and the rationale for this re-scaling procedure. We also discussed comparisons between re-scaled to raw data and the limitations of this procedure that should aid others who wish to examine these data in more detail or by other analytical methods. It is our hope that this review will facilitate the use of the ADNI CSF $A\beta_{1-42}$, t-tau and p-tau_{181} datasets by other biomarker investigators. For studies of ADNIGO+2 subjects that aim to assess BASELINE-only CSF $A\beta_{1-42}$, t-tau and p-tau_{181} data for predictive performance we recommend combining the data for the different subjects across the four datasets(UPENNBIOMK5-8), taking the first occurring BASELINE data for each subject. For studies of longitudinal changes in CSF $A\beta_{1-42}$, t-tau and p-tau_{181} we recommend use of the UPENNBIOMK 6 dataset together with the subjects not included in the latter whose CSF samples were analyzed as part of the UPENNBIOMK4 dataset. This provides longitudinal samples for the ADNI study with the longest trajectories.

Table 5. Recommended datasets for studies using ADNI1 or ADNIGO+2 datasets.

Study	ADNI1	ADNIGO+2
BASELINE-only	UPENNBIOMK	UPENNBIOMKs5-8, taking the first occurring BL data for each subject
Longitudinal changes	UPENNBIOMK6 + subjects not included in the latter whose CSF data are included in UPENNBIOMK4	Only BL + Yr2 currently available, Yr4 in at least 90 subjects collected and will be analyzed in ADNI3

References

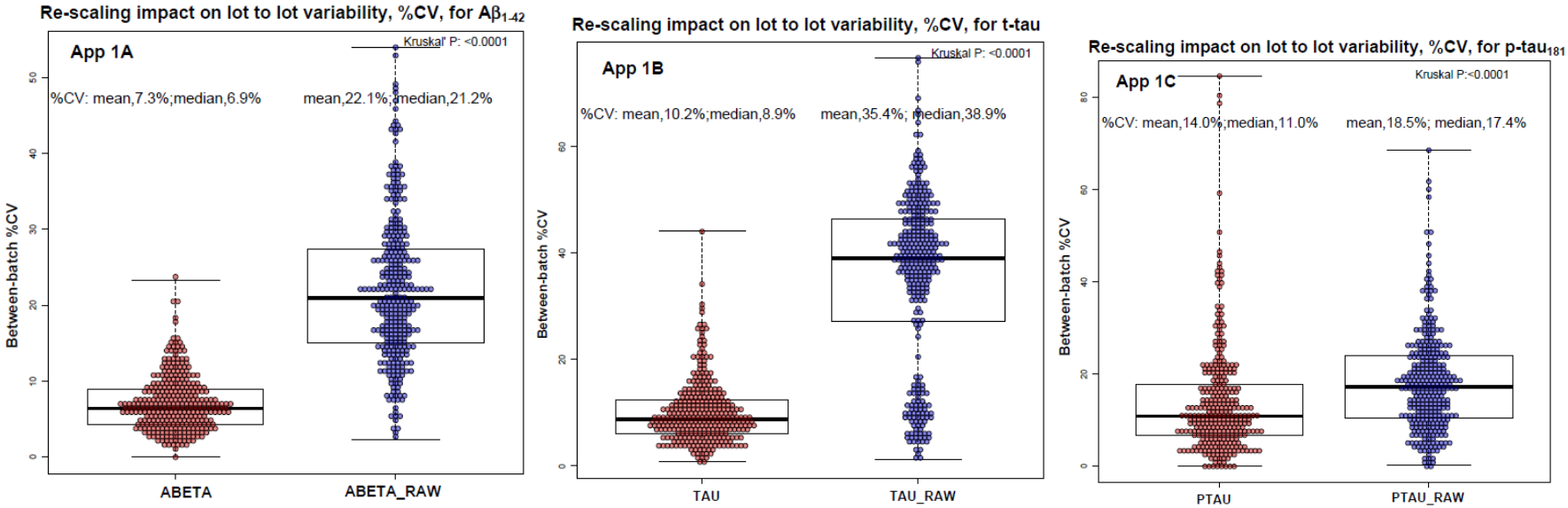
1. Olsson A, Vanderstichele H, Andreasen N, DeMeyer G, Wallin A, Holmberg B, Rosencren L, Vanmechelen E, Blennow K. Simultaneous measurement of $A\beta_{1-42}$, total tau and phosphorylated tau(thr^{181}) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005; 51:336-345.
2. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter W, Lee VM, Trojanowski JQ, ADNI Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Annals of Neurology. 2009;65(4):403-13. PubMed PMID: 19296504.
3. Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Blennow K, Soares H, Simon AJ, Lewczuk P, Dean RA, Siemers E, Potter W, Lee VM, Trojanowski JQ, ADNI Initiative. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathologica. 2011;121(5):597-609. PubMed PMID: 21311900.
4. De Meyer G, Shapiro F, Vanderstichele H, Vanmechelen E, Engelborghs S, De Deyn PP et al. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. Arch Neurol 2010;67:949-956.
5. Okonkwo O, Alosco ML, Griffith HR, Mielke MM, Shaw LM, Trojanowski JQ, Tremont G, and the Alzheimer's Disease Neuroimaging Initiative: Cerebrospinal fluid abnormalities and rate of decline in everyday function across the dementia spectrum: normal aging, mild cognitive impairment, and Alzheimer's disease. Arch Neurol 2010; 67:688-696.
6. Okonkwo OC, Mielke MM, Griffith HR, Moghekar AR, O'Brien RJ, Shaw LM, Trojanowski JQ, Albert MS. the Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid profiles and prospective course and outcome in patients with amnestic mild cognitive impairment. Arch Neurol 2011; 68:113-119.
7. Toledo JB, Brettschneider J, Grossman M, Arnold SE, Hu WT, Xie SX, Lee VM-Y, Shaw LM, John Q. Trojanowski. CSF biomarkers cutoffs: the importance of coincident neuropathological diseases. Acta Neuropathol 2012;124:23-35.
8. Landau SM, Lu M, Joshi AD, Pontecorvo M, Mintun MA, Trojanowski JQ, Shaw LM, Jagust WJ. Comparing positron emission tomography imaging and cerebrospinal fluid measurements of beta-amyloid. Ann Neurol 2013;74:826-836.
9. Toledo JB, Bjerke M, Da X, Landau SM, Foster NL, Jagust W, Jack C, Weiner M, Davatzikos C, Shaw LM, Trojanowski JQ. Nonlinear association between cerebrospinal fluid and florbetapir F-18 Aβ measures across the spectrum of Alzheimer's disease. JAMA Neurol 2015;72: 571-581.
10. Mattsson N, Insel PS, Donohue M, Jagust W, Sperling R, Aisen P, Weiner MW, ADNI. Predicting reduction of cerebrospinal fluid β-amyloid 42 in cognitively normal healthy controls. JAMA Neurol 2015; doi:10.1001, published online March 16, 2015.
11. Petersen RC, Aisen PS, Beckett L, Donohue MC, Gamst AC, Harvey DJ, Jack CR, Jagust WJ, Shaw LM, Toga AW, Trojanowski JQ, Weiner MW. Alzheimer's Disease Neuroimaging Initiative(ADNI): Clinical characterization. Neurology 2010; 74:201-209.
12. Lo RY, Hubbard AE, Shaw LM, Trojanowski JQ, Petersen RC, Aisen PS, Weiner M, Jagust WJ. Longitudinal change of biomarkers in cognitive decline. Arch Neurol 2011;68:1257-1266.
13. Toledo JB, Xie SX, Trojanowski JQ, Shaw LM. Longitudinal change in CSF Tau and Aβ biomarkers for up to 48 months in ADNI. Acta Neuropathol 2013;126:659-70.
14. Mattsson N, Andreasson U, Persson S, Arai H, Batish SD, Bernardini S, Bocchio-Chiavetto L, Blankenstein MA, Carrillo MC, Chalbot S, Coart E, Chiasserini D, Cutler N, Dahlfors G, Duller S, Fagan AM, Forlenza O, Frisoni GB, Galasko D, Galimberti D, Hampel H, Handberg A, Heneka MT, Herskovits AZ, Herukka SK, Holtzman DM, Humpel C, Hyman BT, Iqbal K, Jucker M, Kaeser SA, Kaiser E, Kapaki E, Kidd D, Klivenyi P, Knudsen CS, Kummer MP, Lui J, Llado A, Lewczuk P, Li QX, Martins R, Masters C, McAuliffe J, Mercken M, Moghekar A, Molinuevo JL, Montine TJ, Nowatzke W, O'Brien R, Otto M, Paraskevas GP, Parnetti L, Petersen RC, Prvulovic D, de Reus HP, Rissman RA, Scarpini E, Stefani A, Soininen H, Schroder J, Shaw LM, Skinningsrud A, Skrogstad B, Spreer A, Talib L, Teunissen C, Trojanowski JQ, Tumani H, Umek RM, Van Broeck B, Vanderstichele H, Vecsei L, Verbeek MM, Windisch M, Zhang J, Zetterberg H, Blennow K. The Alzheimer's Association external

quality control program for cerebrospinal fluid biomarkers. Alzheimers Dement 2011;7:386-95.

15. Verwey NA, Bouwman FH, van der Flier WM, Veerhuis R, Scheltens P, Blankenstein MA. Variability in longitudinal cerebrospinal fluid tau and phosphorylated tau measurements. Clin Chem Lab Med 2008; 46:1300-1304.
16. Mattsson N, Insel PS, Donohue M, Landau S, Jagust WJ, Shaw LM, Trojanowski JQ, Zetterberg H, Blennow K, Weiner MW for the Alzheimer's Disease Neuroimaging Initiative. Independent information from cerebrospinal fluid amyloid-β and florbetapir imaging in Alzheimer's disease. Brain 2015; 138:772-83.
17. Dean RA, Shaw LM, Waligorska T, Figurski M, Korecka M, Trojanowski JQ, Sundell KL, Andersen SW, Holdridge KC, Lachno R, Talbot JA, Schuh KJ and Siemers ER. Inclusion of patients with Alzheimer's Disease Pathology in Solanezumab EXPEDITION 3 using Florbetapir (18F) PET imaging or INNO-BIA AlzBio3 CSF $A\beta_{1-42}$. Alzheimer's Dement 2014; 10:Supplement 4:p811.

APPENDIX

App. Figure 1. Dot plots for the distribution of %CV values for all longitudinal replicate samples for ADNI1, ADNIGO and ADNI2 subjects with 2 or more replicate analyses run one year or more apart. **A.** %CV results for CSF $A\beta_{1-42}$ re-scaled as described in the text and for the raw data. **B.** %CV results for CSF t-tau, re-scaled and raw data. **C.** %CV results for CSF p-tau_{181}, re-scaled and raw data.



App. Table 1. %CV of replicates by sample and by number of replicates.

# replicates	VISIT	N	Mean %CV	Median %CV	95% CI
3	Baseline	39	6.5%	6.6%	1.6-12.6%
4	Baseline	89	7.3%	6.5%	2.1-14.6%
5	Baseline	30	8.3%	8.4%	2.3-13.4%
3	12 months	87	6.6%	6.2%	1.7-14.3%
4	12 months	34	8.1%	7.4%	3.4-16.2%
3	24 months	28	7.1%	6.8%	2.8-16.6%
3	36 months	12	8.3%	7.0%	1.6-17.6%

AppTable 2. ADNI CSF batch analyses summary							
CSF batch	Samples tested	Study Phase	Analyses dates, Quarter	Report date, Quarter	re-scaling equation, $A\beta_{1-42}$	re-scaling equation, t-tau	re-scaling equation, p-tau$_{181}$
UPENNBIOMK	410 BL	ADNI1	4th, 2007	1st, 2008	--	--	--
UPENNBIOMK2	410 BL; 328 Yr1	ADNI1	4th, 2008	1st, 2009	Y=1.3X-21.9	Y=0.966X+5.3	Y=0.60X+0.93
UPENNBIOMK3	91 BL; 91 Yr1; 91 Yr2 or 3	ADNI1	3rd, 2009	4th, 2009	--	--	--
UPENNBIOMK4	142 BL;142 Yrs 2-4 follow up	ADNI1	1st,'09-1st,'11	3rd, 2012	Multiple lots*	Multiple lots*	Multiple lots*
UPENNBIOMK5rev	390 BL	ADNIGO/2	1st, 2012	4th, 2013	Y=1.56X-36.6	Y=0.893X+2.6	Y=0.524X+3.6
UPENNBIOMK6	368 BL; 324 ADNI1&2 long	ADNI1/GO/2	1st, 2013	2nd, 2013	Y=1.56X-36.6	Y=0.893X+2.6	Y=0.524X+3.6
UPENNBIOMK7	275 BL; 153 2 or 2.5 Yr	ADNIGO/2	2nd, 2014	3rd, 2014	Y=1.86X-60.9	Y=0.783X+8.3	Y=0.497X+2.7
UPENNBIOMK8	113 BL; 112Yr2;5 2Yr; 1 BL	ADNIGO/2	1st, 2015	2nd, 2015	Y=1.51X-37.8	Y=0.787X+6.5	Y=0.411X+7.6

The specific lot #'s of the AlzBio3 immunoassay kits used in each CSF batch are included in the ADNI "All UPENNBIOMKs" master dataset .csv file, uploaded at the same time as this Methods report on the ADNI/LONI website.

*For UPENNBIOMKs 3 and 4 multiple lot numbers were used over the ~3 year time period for these CSF analyses and a re-scaling equation was generated for each in order to enable re-scaling to ADNI1 BASELINE as described. See Figure App2 A-H below for details for UPENNBIOMK4.

NOTE: As discussed in ADNI CSF BATCH2 below the re-scaled CSF $A\beta_{1-42}$, t-tau and p-tau$_{181}$ values are obtained from the respective re-scaling linear regression equations(obtained from linear regression plots of the respective Batch-specific raw vs original ADNI1 BASELINE results) by solving for X in the re-scaling equation, where Yi is the raw result and Xi is the re-scaled result. For example, for BATCH2 $A\beta_{1-42}$: Xi = Yi-(-21.9)/1.3.

App. Figure 2. App. Figure 2. Panels A,B,C,D,E. Passing-Bablok(P-B) linear regression plots of RAW $A\beta_{1\text{-}42}$ data from UPENNBIOMK4(2009-2011) BASELINE CSF analyses vs the original BASELINE data from UPENNBIOMK(2007). F, G, H, composite P-B plots of re-scaled 2009-2011 datasets vs 2007 original BASELINE for Ab1-42, t-tau and p-tau181, respectively.

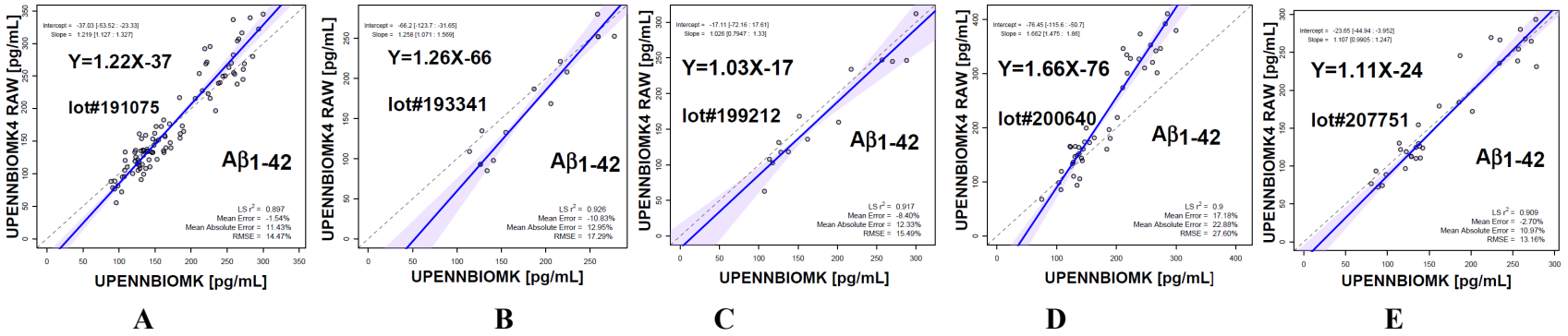
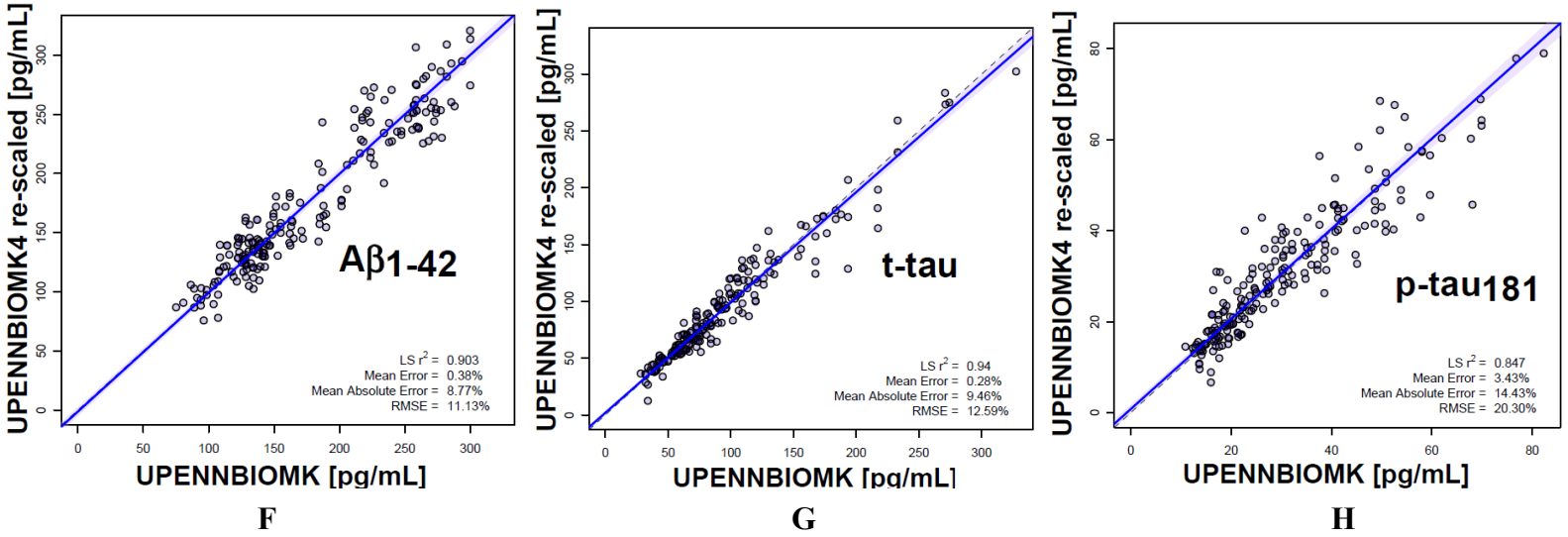
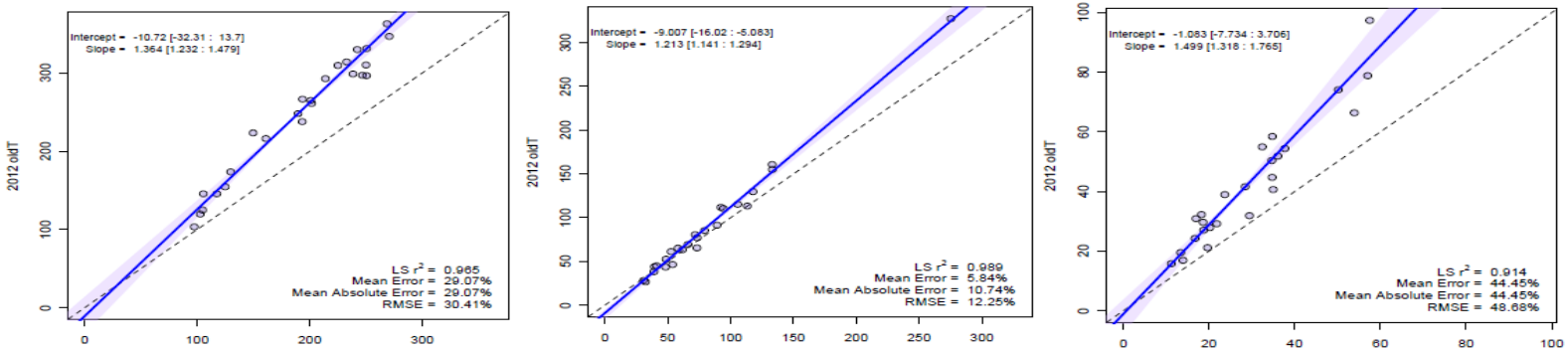


Figure 2. Panels F,G,H. P-B linear regression plots of re-scaled UPENNBIOMK4 datasets vs 2007 original BASELINE dataset. The data in each figure below are all BASELINE UPENNBIOMK4 re-scaled data, obtained from re-scaling analyses as shown above for $A\beta_{1\text{-}42}$, vs corresponding original 2007 BASELINE data for $A\beta_{1\text{-}42}$(F), t-tau(G) and p-tau_{181}(H).

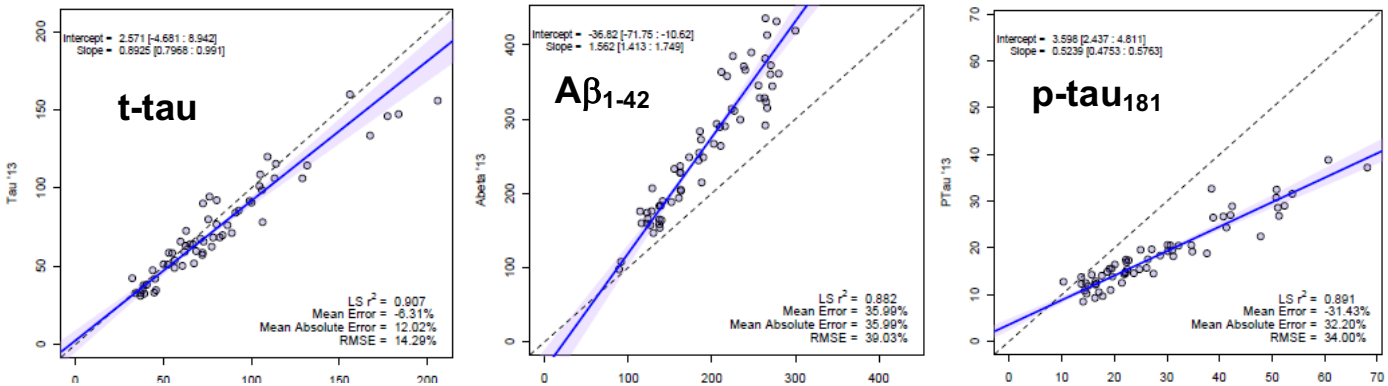


App. Figure 3. Performance assessment of 12-sample re-scaling: comparison of 25 CSF samples assayed and transformed in 2012 using 12 selected ADNI1 BASELINE CSF samples analyzed in one analytical run ("2012 T12") vs 2013 re-scaled that were re-scaled using 62 ADNI I BASELINE CSF samples ("2013 re-scaled 62"). In the 12-sample re-scaling procedure, the selected 12 ADNI1 BASELINE replicate samples were analyzed in one analytical run, and the Passing-Bablok (P-B) linear regression analyses below show that for Aβ1-42, t-tau and p-tau$_{181}$ this highly limited procedure under-re-scaled these CSF samples to the ADNI1 BASELINE samples.



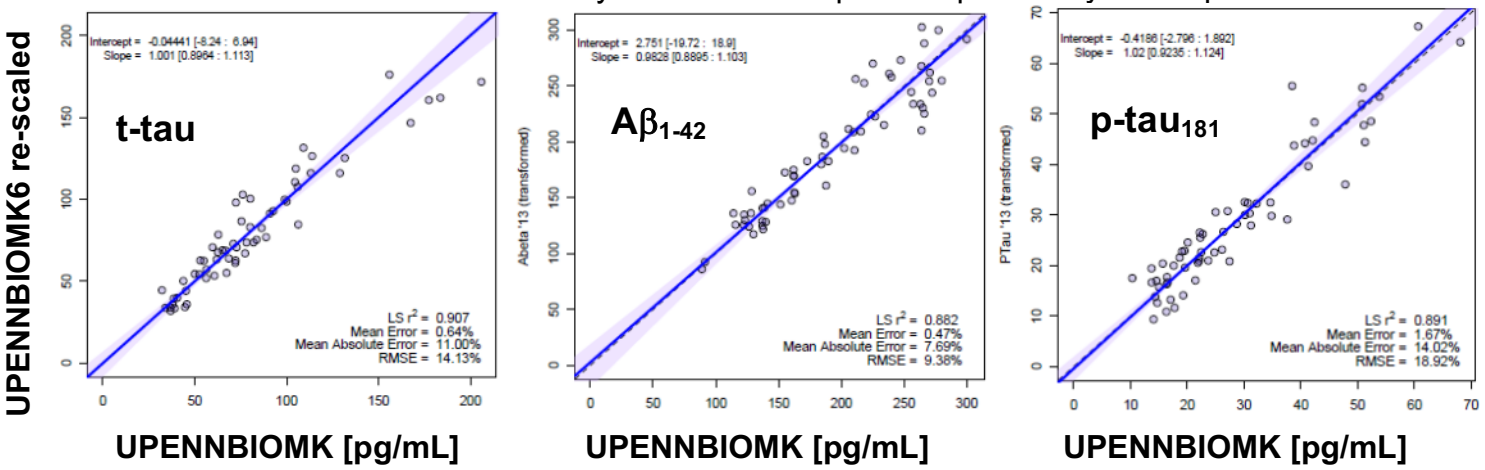
Aβ$_{1-42}$ 2013 re-scaled 62 **t-tau 2013 re-scaled 62** **p-tau181 2013 re-scaled 62**

App. Figure 4A. Re-scaling UPENNBIOMK6 dataset in 2013 to ADNI1 BASELINE involved P-B linear regression analysis of the 62 pristine replicate ADNI BASELINE CSF samples included in the 2013 batch run vs the results for the original analyses in UPENNBIOMK for Aβ$_{1-42}$, t-tau and p-tau$_{181}$.



UPENNBIOMK [pg/mL] **UPENNBIOMK [pg/mL]** **UPENNBIOMK [pg/mL]**

App. Figure 4B. The P-B linear regression plots of CSF re-scaled $A\beta_{1-42}$, t-tau and p-tau$_{181}$ (Y axis), (the 2013 data re-scaled using the P-B linear regression parameters defined by P-B regression analyses in Fig4A and included in App Table1 above, vs ^DNI 1 BASELINE 2007 results for sixty-two ADNI 1 aliquot samples analyzed as part of UPENNBIOMK6.



App. Figure 4C. Shown below are 3 P-B linear regression plots of the twenty-five CSF $A\beta_{1-42}$, t-tau and p-tau$_{181}$ results from 2012 ("UPENNBIOMK5 re-scaled" Y axis) vs ("UPENNBIOMK6 re-scaled", X axis). Each untransformed concentration value was transformed using the P-B linear regression parameters defined in App. Figure 4A above. These re-scaled results confirm the very good agreement between 2012 biomarker concentrations, anchored to ADNI 1 BASELINE as described in the text, for each of the three biomarkers

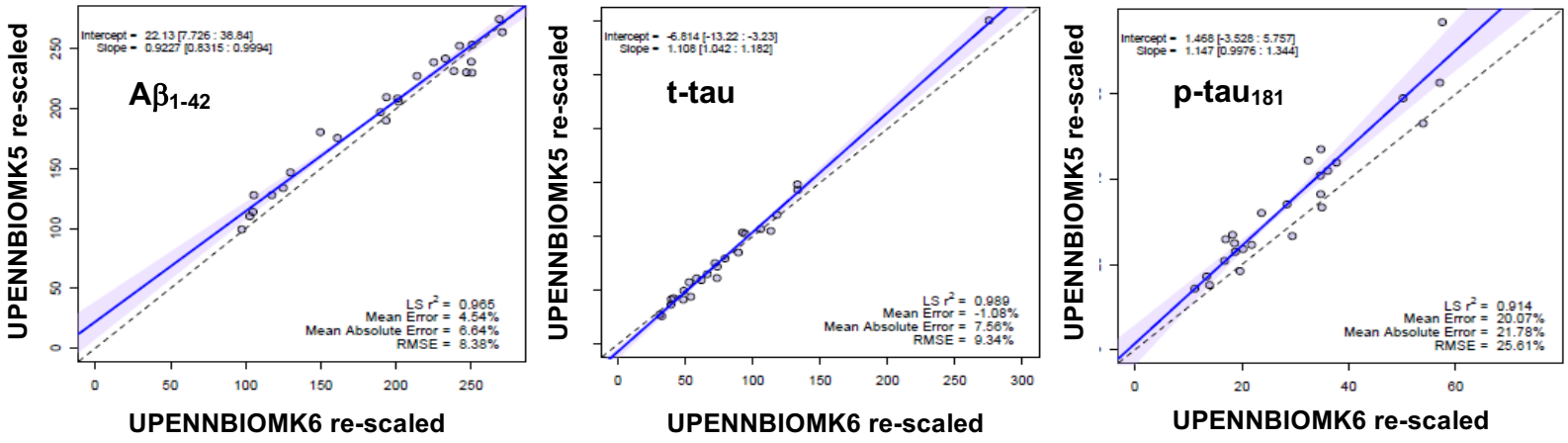


Table 3. . P-B linear regression equation parameters defined by anchoring 2013 analyses of sixty-two ADNI 1 CSFs to 2007 BASELINE data (FigApp. 4B). Ninety-five % confidence intervals are provided for the slope and intercept values.

	$A\beta_{1-42}$	t-tau	$p\text{-}tau_{181}$
P-B reg eqn	Y = 0.98X + 2.75	Y = 1.001X - 0.044	Y = 1.02X - 0.47
Y intercept	2.751(-19 - 18.9)	-0.044(-8.2 - 6.9)	-0.419(-2.8 - 1.8)
Slope	0.983(0.89 - 1.10)	1.001(0.90 - 1.11)	1.02(0.92 - 1.12)
r^2	0.88	0.91	0.89

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