ADNI Alzheimer's Disease Neuroimaging Initiative

An Overview of the Roche Elecsys ADNI CSF Batch Analyses run in ADNI3

Leslie M Shaw, Magdalena Korecka, Yang Wan, Alexandria Sciulli, Eddie B Lee, Magdalena Brylska

Department of Pathology & Laboratory Medicine and Center for neurodegenerative Diseases Research, Perelman School of Medicine University of Pennsylvania (UPENN)

EXECUTIVE SUMMARY

In preparation for the ADNI3 study the Biomarker Core together with ADNI leadership including the Executive Committee and the PPSB Biofluid Biomarker Working Group discussed and reviewed ongoing developments to improve on the performance of the then available Research Use Only immunoassays including the INNA-BIA AlzBio3 Immunoassay, the immunoassay used for analyses of CSFs collected during the ADNI1 and ADNIGO/2 study phases. At the time (2014-2015), several companies had been developing "next generation" immunoassays for CSF Aβ42, t-tau and p-tau181, some of which are fully automated. After systematic reviews and discussion of the available immunoassays and available performance data at the time, the decision was taken to implement the fully automated Roche Elecsys® immunoassay for analysis of these CSF AD biomarkers in all available ADNI1/GO/2 CSF samples and subsequently in all ADNI3 CSF samples. Amongst the sought after improvements over RUO immunoassays were: reduced number of as many manual steps as possible; improved precision and accuracy performance within and especially between laboratories; improved reagent lot-to-lot performance for the immunoassay kits. Achievement of these improvements was expected to enable IVD test approval, use in clinical practice, and result in the ability to use these biomarker tests in treatment trials to accurately identify patients who have AD pathology for inclusion into the trials - especially in the international setting where local laboratory support is essential.

This Methods report provides an overview for the series of 5 datasets for CSF Aβ42, Aβ40 (in ADNI3 CSFs), t-tau and p-tau181 analyses reported by the ADNI Biomarker Core from 2016 through 2022 on the ADNI/LONI website. Accompanying this report is a .CSV datafile, "**UPENN CSF Biomarkers Elecsys (BATCH 9-13) [ADNI1, GO, 2, 3]**". The first and largest dataset UPENNBIOMK9, reported 12/2017, contained CSF Aβ42, t-tau and p-tau181 results for 2,401 pristine CSF aliquot samples from ADNI1/GO/2 participants. This was followed by reports of analyses for subsequent batches of CSF aliquot samples from ADNI3 participants summarized in the Table below. Aβ40 was

included in all batches except the first one due to reagent availability, only from late 2017, as a research test.

Table 1. Batches of ADNI CSF and report dates performed during ADNI3.

Batch name	Report Date	CSF samples	Analytes	Batch ID
BIOMK9	4/2017	2401 ADNI1/GO/2	Aβ42, t-tau, ptau181	ADNI1/GO/2 batch
BIOMK11	6/2019	498 ADNI3	Aβ42/40, t-tau, ptau181	ADNI3 1st batch
BIOMK12	8/2020	216 ADNI3	Aβ42/40, t-tau, ptau181	ADNI3 2nd batch
BIOMK13a & b	12/2022	216 ADNI3; 89 ADNI3	Aβ42/40, t-tau, ptau181	ADNI3 3rd & 4th batches
*NOTE there are 133 DoD CSF results not included in the "UPENN CSF Biomarkers Elecsys Methods (Batch 9-13) [ADNI1,GO,2,3]" dataset. They are reported separately in a DoD/ADNI file.				

Methods and Procedures

The Roche Elecsys β-Amyloid(1-42) CSF, Elecsys β-Amyloid(1-40) CSF, Elecsys Total-Tau CSF, and Elecsys Phospho-Tau(181P) CSF immunoassays were used following a Roche Study Protocol at the UPenn/ADNI Biomarker Laboratory, according to the kit manufacturer's instructions and as described in previous studies (1-3). Analyses were performed in a series of runs, each sample run one time (in singlicate) for each of the 3, or 4, biomarker tests, and reported at the times provided in the above Table. Following the initial batch (BIOMK9) each subsequent batch of ADNI3 samples included a standard new lot rollover protocol from the manufacturer that was conducted over a 10 working day timeline and involved repeated analyses of quality control samples. Acceptance criteria as documented according to the Roche Protocol in the UPenn/ADNI Biomarker Laboratory were followed for these analyses. The precision performance was further documented by inclusion of two CSF pools, one abnormal and one normal, each created from residual CSF leftover from analyses of ADNI CSFs.

BIOMK9 Batch analyses of 2,401 ADNI1/GO/2 CSF samples.

A total of 2401 never before thawed aliquots of ADNI1, ADNIGO/2 CSF samples collected between 9/7/2005 and 7/25/2016, (collection date for each aliquot sample provided in the **UPENN CSF Biomarkers Elecsys (BATCH 9-13) [ADNI1,GO,2,3]** dataset**)**, were analyzed by the electrochemiluminescence immunoassays (ECLIA) Elecsys β-Amyloid(1-42) CSF, Phospho-Tau(181P) CSF, and Total-Tau CSF on a fully automated Elecsys **cobas e** 601 instrument and a single lot of reagents for each of the 3 measured biomarkers. At the time performed these immunoassays were for investigational use only. They were under development by Roche Diagnostics and not commercially available at the time performed.

Method.

The Roche Elecsys β-Amyloid(1-42) CSF, Elecsys Total-Tau CSF, and Elecsys Phospho-Tau(181P) CSF immunoassays were used according to the preliminary kit manufacturer's instructions and as described in previous studies (1,2). Analyses were performed in a series of 36 runs, each sample run one time (in singlicate) for each of the 3 biomarker tests, over the time period of November 17, 2016 through January 20, 2017. Acceptance criteria as documented according to the Roche Protocol in the UPenn/ADNI Biomarker Laboratory were followed for these analyses.

In each of the 36 analytical runs, quality control results were within stated limits to meet acceptance criteria for precision and accuracy (detailed data in "ADNI1, GO and 2 CSF report: 2017"). The analyte measuring ranges were, lower technical limit to upper technical limit for each biomarker: 200 to 1700 pg/mL for Elecsys β-Amyloid (1-42) CSF immunoassay, 80 to 1300 pg/mL for the Elecsys Total-Tau CSF immunoassay and 8 to 120 pg/mL for Elecsys Phospho-Tau (181P) CSF immunoassay. For results that are above the upper technical limit, the result is stated as ">" the respective upper technical limit values or if below the lower technical limit, the result is stated as "<" the respective lower technical limit value in the "**UPENN CSF Biomarkers Elecsys (BATCH 9-13) [ADNI1,GO,2,3]"** dataset.

Exploratory Elecsys β-Amyloid(1-42) CSF immunoassay measurement results above the technical limit of 1700 pg/mL were provided by Roche Diagnostics based on an extrapolation of the calibration curve and are shown in a clearly marked separate column in the ***"* UPENN CSF Biomarkers Elecsys (BATCH 9-13) [ADNI1,GO,2,3]"** dataset.

Please note:

The Elecsys β-Amyloid(1-42) CSF immunoassay used during the ADNI3 phase was not a commercially available IVD assay. It is an assay that was under development and for investigational use only. The measuring range of the assay is 200 (lower technical limit) – 1700 pg/mL (upper technical limit). The performance of the assay beyond the upper technical limit was not formally established. Therefore use of values above the upper technical limit, which are provided based on an extrapolation of the calibration curve, is restricted to exploratory research purposes and is excluded for clinical decision making or for the derivation of medical decision points.

Investigators should include the above disclaimer in any publication using Elecsys β-Amyloid(1-42) CSF immunoassay values above the upper technical limit.

It should also be noted that values above the measuring range for a particular sample may differ from concentration values measured by any potential future Elecsys β-Amyloid (1-42) CSF immunoassay assay.

As part of the validation process for the $A\beta_{1-42}$ test method, Roche conducted collaborative studies of comparisons between the Elecsys β-Amyloid (1-42) CSF

immunoassay and two LC/MSMS-based reference methods (3,4) certified by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) (1-4).

The frequency distributions for the measurement results of the described Elecsys immunoassays for Aβ42/Aβ40 ratio are shown in Figure 1 for ADNI3 participants showing the bimodal distribution that characterizes this biomarker test. The strong correlation between t-tau and p-tau$_{181}$ for 1215 BASELINE CSF samples from ADNI1/GO/2 patients is shown in Figure 2.

Please note that due to the sticky properties of Aβ$_{1-42}$, the absolute measured concentrations of Aβ$_{1-42}$ are affected by pre-analytical handling procedures, including the specific type and volume (in relationship to CSF volume) of plastic tubes used, the number of transfer steps, and the number of freeze-thaw steps. To better understand possible differences in CSF Aβ$_{1-42}$ levels measured in studies that use different pre-analytical handling procedures, detailed direct comparison between pre-analytical procedures, and statistical methods utilized will be required.

Figure 1. Frequency distribution histogram plot for CSF Aβ42/Aβ40 for all ADNI3 CSFs using the Roche Elecsys immunoassay on a **cobas e** 601 instrument. Please note that only Aβ42/Aβ40 ratio values are included wherein the Aβ42 results were within the measuring range for this analyte. The red curves were generated using mixture modeling, a statistical technique shown for the ADNI and other studies to provide a disease-independent approach to defining cutpoints for CSF AD biomarkers(6,7,8). This method fits two Gaussian normal distribution curves on the assumption that the biomarker distributions can be described by two subpopulations one an Alzheimer's Disease (AD) sub-population and the other a non-AD sub-population each defined by the presence or absence of an AD biomarker signature. Using this methodology for deriving cutpoints a value of 0.061 was determined for the Aβ42/Aβ40 ratio.

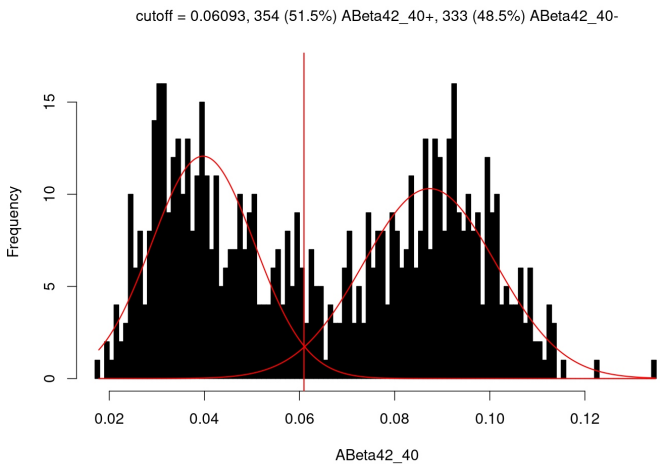
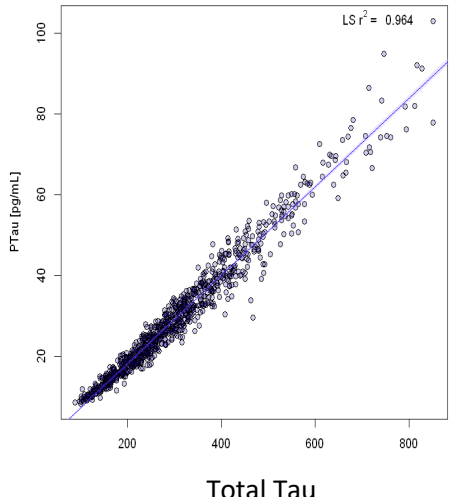


Figure 2. Correlation analysis for p-tau$_{181}$ vs t-tau for 1215 ADNI1/GO/2 subjects' BASELINE CSF aliquot samples measured with the Roche Elecsys® immunoassays on a cobas e 601 instrument.



BIOMK11-13 Batch analyses of ADNI3 CSF samples.
A total of 1,030 CSF samples obtained from ADNI3 participants were analysed and reported in Batches from 6/2019 to 12/2022. The analyses were performed as described above for BIOMK9 with the addition of a "lot rollover" protocol as summarized above and two QC pool aliquots were included in each daily run with results summarized in Table 2 below.

Table 2. Precision performance for ADNI3 CSF sample analyses using CSF pool samples.

BMK11	N	Abnormal	Normal	BMK13a	N	Abnormal	Normal
Aβ42	12	511±16(3.2%)	982±35(3.6%)	Aβ42	5	544±9(1.7%)	956±32(3.4%)
Aβ40	12	12253±364(3.0%)	13440506(3.8%)	Aβ40	5	10360±174(1.7%)	11450±150(1.3%)
pTau181	12	42.20.6(1.3%)	15.80.2(1.4%)	pTau181	5	24.1±0.3(1.1%)	13.6±0.2(1.8%)
t-tau	12	555±7.8(1.4%)	188±2.8(1.5%)	t-tau	5	314±2.5(0.8%)	182±1.7(0.9%)
BMK12				**BMK13b**			
Aβ42	3	515±21(4.1%)	1010±9(0.85%)	Aβ42	4	546±16(2.9%)	962±55(5.7%)
Aβ40	3	10150±186(1.8%)	12300±278(2.3)	Aβ40	4	10210±322(3.2%)	11345±252(2.2%)
pTau181	3	25.10.12(0.48%)	15.80.06(0.39%)	pTau181	4	23±0.4(1.9%)	13.6±0.2(1.8%)
t-tau	3	320±2.4(0.76%)	170±0.8(0.47%)	t-tau	4	312±3.9(1.2%)	178±2.4(1.4%)
NOTE:the CSF pools used were the same for BMK12, 13a and 13b.Two different pools were available for BMK 11.							

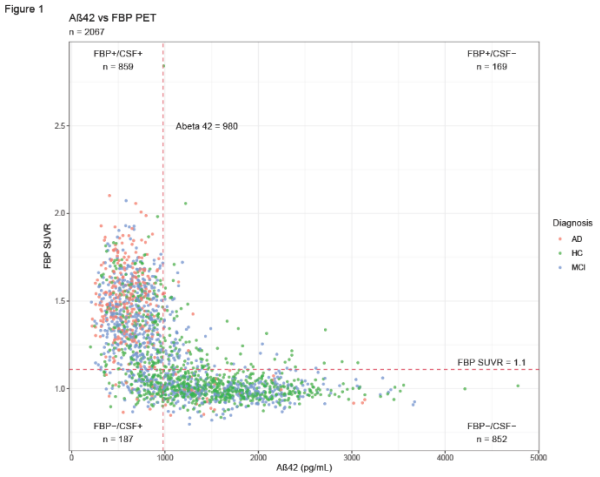
BATCH to BATCH PERFORMANCE for BIOMK11, BIOMK12, BIOMK13a and 13b.
In order to document BATCH-to-BATCH performance of the Roche Elecsys Immunoassay reagents, each BATCH subsequent to BATCH9 (BATCH9 included all ADNI1/GO/2 CSFs), some BATCH9 CSF samples were included in the BIOMK11, 12 and 13a and 13b BATCH analyses to allow for direct comparisons across the new BATCHes to the BATCH9 performance assuming this BATCH9 to be the reference for subsequent ADNI3 BATCHes. Since BIOMK9 did not include Aβ40 testing we could not use BIOMK9 for reference for that analyte. Overall the results were excellent thus precluding the need for "re-scaling" each subsequent batch. This result was the expected outcome for an FDA approvable test system. The actual test/retest data are summarized below in Appendix A, Table APP1, of this report.

COMPARISON OF ADNI CSF AD BIOMARKER TO FLORBETAPIR PET RESULTS.
The below plots (Figures 3, 4 and 5) compare FBP PET with CSF Aβ42; CSF ptau181/Aβ42 and to CSF Aβ42/Aβ40, respectively. Concordance values are summarized in Table 3 below. The respective cutpoints used for these analyses are: Aβ42, 980 pg/mL; ptau181/Aβ42, 0.025 and Aβ42/Aβ40, 0.061; FBP PET SUVR, 1.11. Since Aβ42/Aβ40 data are only available in ADNI3 CSFs the analyses involving this ratio are restricted to ADNI3 CSF data only. These analyses were repeated using the Centiloid units for FBP analysis results with the same overall results for concordance and for AUC values. AUC values obtained are 0.894, 0.952 and 0.950 respectively:

Figures.

Figure 3. FBP SUVR vs CSF Aβ42

Figure 4. FBP SUVR vs CSF ptau181/Aβ42



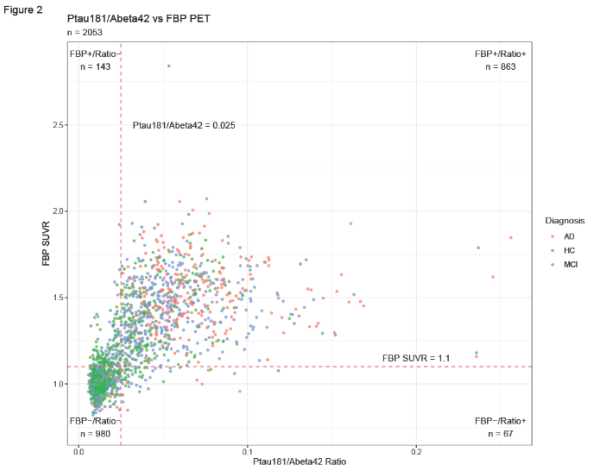
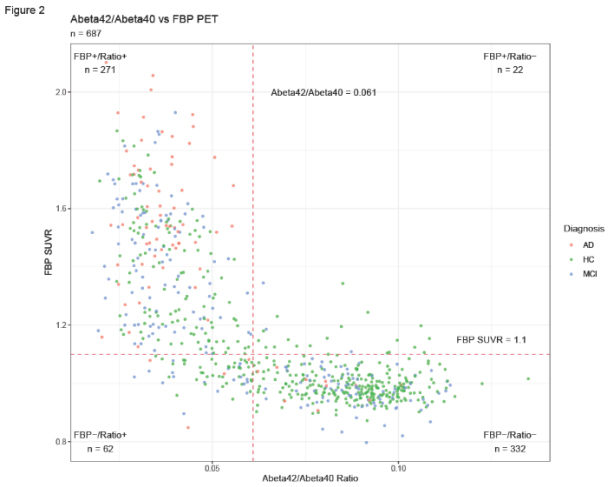


Figure 5. FBP SUVR vs CSF Aβ42/Aβ40

Figure 6. FBP SUVR vs CSF t-tau/Aβ42



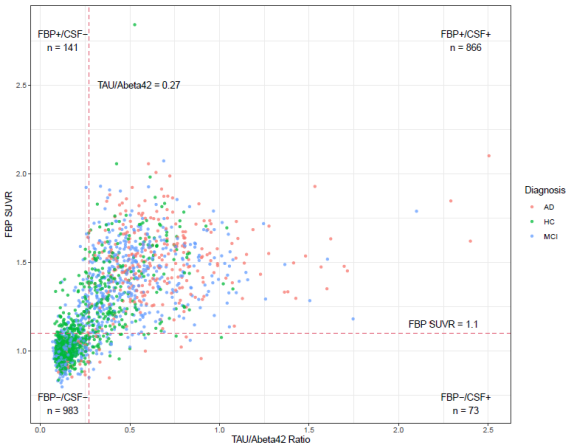


Table 3. concordance between Florbetapir PET and CSF Aβ42; CSF ptau181/Aβ42 and to CSF Aβ42/Aβ40.

	N	PET+/CSF+	PET-CSF-	PET+/CSF-	PET-/CSF+	Concord	Discord	AUC	Sens/Spec
Aβ42	**2067**	**859**	**852**	**169**	**187**	**82.7%**	**17.2%**	**0.894**	**0.82/0.84**
p-tau181/Aβ42	**2063**	**869**	**965**	**158**	**61**	**89.3%**	**10.7%**	**0.952**	**0.90/0.91**
t-tau/Aβ42	**2063**	**866**	**983**	**141**	**73**	**89.6%**	**10.4%**	**0.950**	**0.89/0.92**
Aβ42/Aβ40	**687**	**271**	**332**	**22**	**62**	**87.8%**	**12.2%**	**0.950**	**0.89/0.92**
NOTE: for Aβ42 and ptau/Aβ42 all ADNI1/GO/2/3 CSF data used; for Aβ42/Aβ40, ADNI3 CSF data used since Aβ40 data is available for ADNI3 CSFs only.									

RECOMMENDATIONS FOR USE OF DATASETS ACROSS TIME.

For studies of longitudinal concentrations across time for these CSF AD biomarkers and ratios results selection needs to be considered due to the fact that some aliquots were re-tested in subsequent batches as discussed above. We recommend use of the first

measured aliquot sample when a particular individual's aliquot sample at a certain timepoint is being considered.

References

1. Bittner T, Zetterberg H, Teunissen CE, Ostlund RE, Militello M, Andreasson U, Hubeek I, Gibson D, Chu DC, Eichenlaub U, Heiss P, Kobold U, Leinenbach A, Madin K, Manuilova E, Rabe C, Blennow K. Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of β-amyloid (1-42) in human cerebrospinal fluid. Alz Dement 2016; 12: 517-526.
2. Shaw LM, Fields L, Korecka M, Waligorska T, Trojanowski JQ, Allegranza D, Bittner T, He Y, Morgan K, Rabe C. Method comparison of Aβ(1-42) measured in human cerebrospinal fluid samples by liquid chromatography tandem mass spectrometry, the INNO-BIA AlzBio3 assay and the Elecsys®β-amyloid(1-42) assay. AAIC 2016.
3. Leinenbach A, Pannee J, Dulffer T, Huber A, Bittner T, Andreasson U, Gabom J, Zetterberg H, Kobold U, Portelius E, Blennow K. Mass spectrometry-based candidate reference measurement procedure for quanitification of amyloid-β in cerebrospinal fluid. Clin Chem 2014; 60: 987-994. **{C11RMP9}**.
4. Korecka M, Waligorska T, Figurski M, Toledo JB, Arnold SE, Grossman M, Trojanowski JQ, Shaw LM. Qualification of a surrogate matrix-based absolute quantification method for amyloid-β42 in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry. J Alz Dis 2014;41: 441-451. **{C12RMP1}**.
5. Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, Lifke V, Corradini V, Eichenlaub U, Batrla R, Buck K, Rabe C, Blennow K, Shaw LM. CSF biomarkers of Alzheimers disease concord with amyloid- PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. Alzheimers and Dementia 2018; 14: 1470-1481.
6. Royse SK, Minhas DS, Lopresti BJ, Murphy A, Ward T, Koeppe RA, Bullich S, DeSanti S, Jagust WJ, Landau SM, and ADNI. Validation of amyloid PET positivity thresholds in cantiloids: a multisite PET study approach. Alz Res Ther 2021; 13(1):99. doi:10.1186/s13195-021-00836-1
7. DeMeyer G, Shapiro F, Vanderstichele H, Vanmechelen E, Engelborghs S, DeDeyn PP, Coart E, Hansson O, Minthon L, Zetterberg H, Blennow K, Shaw L, Trojanowski JQ. Diagnosis independent Alzheimer Disease biomarker signature in cognitively normal elderly people. Arch Neurol 2010; 67:949-956.
8. Fraley C, Raftery AE, Murphy BT, Scrucca L. mclust Version4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation Technical Report no. 597, Department of statistics, University of Washington.
9. Samtani MN, Raghavan N, Shi Y, Novak G, Farnum M, Lobanov V, Schultz T, etal and the Alzheimer's Disease neuroimaging Initiative. Disease progression model in MCI subjects from Alzheimer's Disease Neuroimaging Initiative: CSF biomarkers predict population subtypes. Br J Clin Pharm 75: 146-161, 2013.

APPENDIX A.

Table APP1. Summary of linear regression analyses of test-re-test CSF samples.

BATCH	#CSF re-tested	Linear regression eqn: Y(BATCH)=m*X(BATCH9) + Y intercept; R^2		
		Aβ42	t-tau	p-tau181
11	89	Y=1.07X-105; R^2=0.98	Y=0.95X+8.4;R^2=0.99	Y=0.99X-0.22;R^2=0.99
12	28	Y=1.01X+29.2;R^2=0.88	Y=0.96X+5.8-R^2=0.99	Y=0.96X-0.69;R^2=0.99
13a	50	Y=1.17X-76;R^2=0.93	Y=0.99X+0.4;R^2=0.99	Y=0.97X-0.44;R^2=0.99
13b	23	Y=1.01X+35;R^2=0.98	Y=0.98X-4.5;R^2=0.99	Y=1.0X-1.9;R^2=0.99
Aβ40 test-re-test data available only for BATCHES 12, 13a and 13b: 12—Y=0.91X+1964, R^2=0.98; 13a—Y=0.94X+0.88, R^2=0.95; 13b—Y=1.01X-0.61; R^2=0.95				

Version Information

This report is a summary of previously reported Roche Elesys data with the purpose of combining together all 6 batch analyses completed during ADNI3 and includes basic performance information such as QC results and batch to batch performance experience. This will hopefully facilitate studies across all BASELINE and longitudinal CSFs collected during Phases1/GO,2 and 3. All of this CSF data is combined into a single dataset ***"UPENN CSF Biomarkers Elecsys (BATCH 9-13) [ADNI1,GO,2,3]".***

About the Authors

This document was prepared by Leslie M Shaw (shawlmj@pennmedicine.upenn.edu; 215-662-6575), Magdalena Korecka, Yang Wan, Alexandria Sciullia, Eddie B Lee and Magdalena Brylska, Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pannsylvania.