ADNI Alzheimer's Disease Neuroimaging Initiative

ADNI 3: Batch analyses of $A\beta_{1-42}$, t-tau and p-tau_{181} in ADNI1 and ADNIGO/2 CSF using the fully automated Roche Elecsys and cobas e immunoassay analyzer system

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Introduction

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 available Research Use Only immunoassays including the INNA-BIA AlzBio3 immunoassay, the immunoassay which had been used for analysis of CSFs collected during the ADNI1 and ADNIGO/2 study phases. Several companies have been developing "next generation" immunoassays for CSF $A\beta_{1-42}$, t-tau and p-tau_{181}, some of which are fully automated. After the 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. Amongst the sought after improvements over RUO immunoassays are: 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 is expected to enable IVD test approval 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. The following is a brief description of the analysis results achieved using the Roche Elecsys immunoassay platform.

Summary

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 UPENNBIOMK9 .CSV file), 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 (provided in UPENNBIOMK9 .CSV file). These immunoassays are for investigational use only. They are currently under development by Roche Diagnostics and not commercially available yet.

A report "ADNI1, GO and 2 CSF:2017" is in preparation that provides details for the analyses including calibrator and quality control results.

Method

The Roche Elecsys β-amyloid(1-42) 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 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 .CSV file "UPENNBIOMK9".

Exploratory Elecsys β-Amyloid(1-42) CSF immunoassay measurement results above the technical limit of 1700 pg/mL will be provided by Roche Diagnostics based on an extrapolation of the calibration curve and will be shown in a clearly marked separate column in the .CSV file "UPENNBIOMK9".

Please note:

> The Elecsys β-Amyloid(1-42) CSF immunoassay in use is not a commercially available IVD assay. It is an assay that is currently 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 has not been 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 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, including $A\beta_{1-42}$ and the $\log_e$ for the two ratios, t-tau/$A\beta_{1-42}$ and p-tau_{181}/$A\beta_{1-42}$ excluding $A\beta_{1-42}$ results that were above or below the measuring ranges, are shown in Figure 1A, 1B, and 1C for ADNIGO/2 SMC+EMCI+LMCI+AD patients, who had concomitant Florbetapir PET β-amyloid imaging testing, showing the bimodal distributions that characterize these biomarker test parameters. Very comparable patterns for these biomarkers are also characteristic of the ADNI1 BASELINE patient groups (data not shown). 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 $A\beta_{1-42}$, the absolute measured concentrations of $A\beta_{1-42}$ 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 $A\beta_{1-42}$ 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. Comparison studies of the pre-analytical handling procedure used in ADNI data to that used in other studies has been initiated and is an ongoing effort for the purpose of understanding the potential contribution of this and other factors in determining cutoff values for $A\beta_{1-42}$, t-tau and p-tau_{181} that can be transferred between studies (5).

Ongoing analyses will further describe the performance characteristics for the Roche Elecsys® for detection of AD pathology

Figure 1. Frequency distribution histogram plots for CSF $A\beta_{1-42}$ alone, and the ratios, t-tau/$A\beta_{1-42}$ and p-tau_{181}/$A\beta_{1-42}$, measured in ADNIGO/2 SMC+EMCI+LMCI+AD, restricted to the subset of patients that also had a BASELINE Florbetapir(FBP) amyloid-β PET scan, using the Roche Elecsys immunoassays on a **cobas e** 601 instrument. Fig 1A. $A\beta_{1-42}$; 1B. $\log_e$ t-tau/$A\beta_{1-42}$; 1C. $\log_e$ p-tau/$A\beta_{1-42}$. Please note that only biomarker concentration values within the respective measuring ranges for $A\beta_{1-42}$, t-tau and p-tau_{181} are included. The red and blue 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 (red curves) and the other a non-AD sub-population (blue curves) each defined by the presence or absence of an AD biomarker signature.

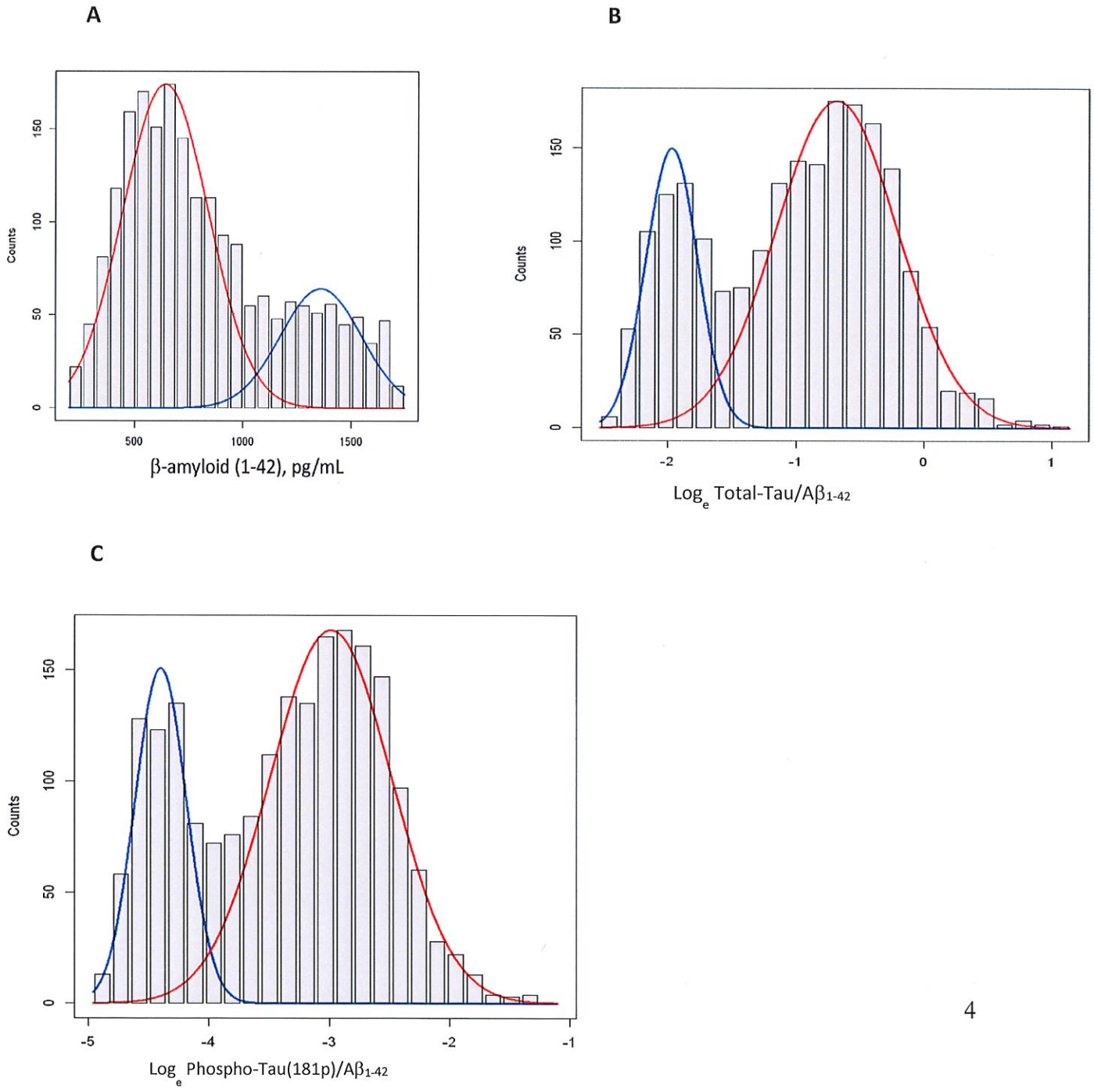
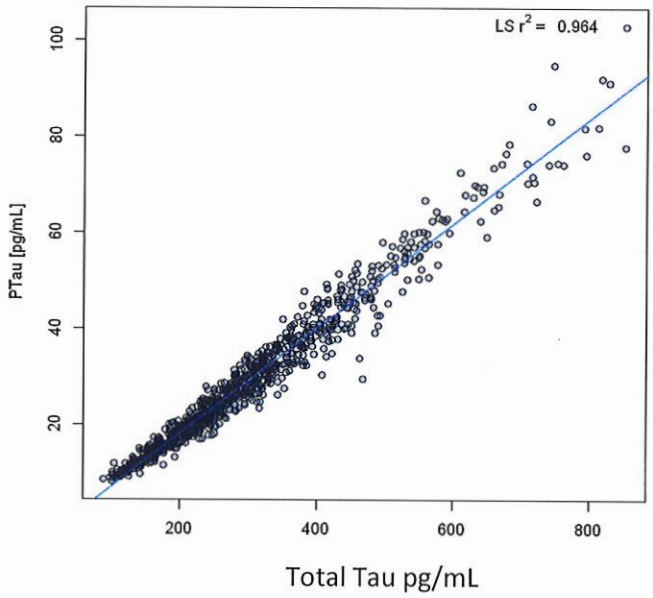


Figure 2. Correlation analysis for p-tau$_{181}$ vs t-tau for 1215 ADNI1/GO/2 subjects' BASELINE CSF aliquot samples measured with the Roche Elecsys® immunoassays on a cobas e 601 instrument.



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 (see Appendix).
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, Blennow K, Trojanowski JQ, Seibyl J, Stomrud E, Bittner T, Rabe C, Eichenlaub U, Shaw LM. Concordance between new Elecsys® CSF assays

(Abeta(1-42), Total Tau and Phosphorylated Tau) and amyloid-PET imaging in BioFINDER and ADNI. ADPD, 2017.
6. 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.
7. 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.
8. 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 Alzheimmer's Disease Neuroimaging Initiative: CSF biomarkers predict population subtypes. Br J Clin Pharm 75: 146-161, 2013.

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