ADNI Alzheimer's Disease Neuroimaging Initiative

ADNI CSF analyses of $A\beta_{1-42}$, $A\beta_{1-40}$, t-tau and p-tau_{181} in the DIAN/ADNI study using the fully automated Roche Elecsys and cobas e 601 immunoassay analyzer system

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Introduction

The ADNI and DIAN(Dominantly Inherited Alzheimer's Network) teams have joined together in the DIAN-ADNI study. As part of this study the ADNI Biomarker core has analyzed and quality controlled 1,089 (BASELINE plus longitudinal) CSF aliquot samples (422 from ADNI participants and 667 from DIAN participants) to determine concentrations of Aβ42, Aβ40, t-tau and p-tau181 using the fully automated Roche Elecsys and cobas e 601 immunoassay analyzer system. The following is a brief description of the analysis results achieved using the Roche Elecsys immunoassay platform, including histogram distributions for the 422 ADNI CSF samples and quality control performance.

As per DIAN protocols, a follow-up report and dataset that will include the results for the 667 CSFs from the DIAN cohort will be posted following internal review of the DIAN dataset by DIAN investigators.

Summary

A total of 422 never before thawed aliquots of ADNI1, ADNIGO/2 CSF samples were analyzed by the electrochemiluminescence immunoassays (ECLIA) Elecsys $A\beta_{1-42}$ CSF, $A\beta_{1-40}$ CSF, p-tau_{181} CSF, and t-tau CSF on a fully automated Elecsys **cobas e** 601 instrument and a single lot of reagents for each of the 4 measured biomarkers (lot# for each analyte: P09, $A\beta_{1-42}$; A01, $A\beta_{1-40}$ and P02 for t-tau and p-tau_{181}). The Roche $A\beta_{1-42}$, p-tau_{181} and t-tau immunoassays are available for clinical use in Europe and awaiting approval in the USA. The $A\beta_{1-40}$ immunoassay is for investigational use only (IUO). Included in this report are histogram frequency distribution plots (Fig 1A-D) for these ADNI CSFs showing bimodal distributions that are characteristic of $A\beta_{1-42}$, the ratio $A\beta_{1-42}/A\beta_{1-40}$ and the ratios, $\log_e$ t-tau/$A\beta_{1-42}$ and $\log_e$ p-$tau_{181}/A\beta_{1-42}$ (3,4,7,8,9). Quality control analyses, including results for an "AD-like" CSF pool (i.e. pooled CSF from subjects with clinical AD or MCI with elevated tau and depressed $A\beta_{1-42}$ levels consistent with autopsy-confirmed AD patients) are summarized showing run to run precision performance (Fig 2). Precision performance from run to run ranged from average values of 1.6% to 3.8% across the 4 biomarkers, achieving the expected tight range for repeated measurements for replicate pristine CSF pool aliquot samples.

Test-re-test performance is shown in Figure 3 for the 422 CSF samples for $A\beta_{1-42}$. For the latter analysis, pristine replicate aliquots of the 422 CSF aliquot samples had also been run as part of analyses of all available ADNI CSF samples November 2016-January 2017 using a different lot of reagents, lot P06. This analysis shows tight correlation between the two datasets with an r^2 value of 0.984, an intercept value of -3.847(95% CI:-12.2 to +4.87), indistinguishable from 0, and a slope of 0.8953(0.8832-0.9078), showing approximately 10% lower values for these more recent analyses. These values, except for the slope value, exceed the criteria for acceptable lot to lot performance recently described by Algeciras-Schimnich(1): [intercept <50% lower than the lowest reportable value; r^2>0.95; slope between 0.90-1.10]. In order to achieve complete comparability of the assay results for $A\beta_{1-42}$ we re-scaled these results for the 422 CSFs to the values measured during the earlier run of pristine replicates, using the regression equation, Xi = Yi – (-3.847)/0.8953 where Xi are the re-scaled values and Yi are the raw values for the 422 CSFs in the current study. Using this approach we achieved a corrected slope value of 0.984(0.9823-1.009), indistinguishable from a value of 1.0, and intercept of +3.77(-5.432-13.18), indistinguishable from a value of 0 and excellent agreement across all tested samples, an r^2 value of 0.984. The comparisons for t-tau and p-tau181 for these 422 CSFs vs the data from the earlier analyses showed by linear regression acceptable performance according to these acceptance criteria. For t-tau, r^2=0.989, intercept=12.03, and slope=0.9977; for $p\text{-}tau_{181}$, r^2=0.991, intercept=0.70, and slope=1.066.

Method

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 preliminary kit manufacturer's instructions and as described in previous studies (2-4). Analyses were performed in a series of runs, each sample run one time (in singlicate) for each of the 4 biomarker tests, over the time period of October 18, 2017 through November 9, 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 14 days of performing analyses, quality control results were within stated limits to meet acceptance criteria for precision and accuracy. There were 2 runs each day of approximately 40 samples each run. The analyte measuring ranges were, lower technical limit to upper technical limit for each biomarker: 200 to 1700 pg/mL for the Elecsys $A\beta_{1-42}$ CSF immunoassay, 22 to 40,300 pg/mL for the Elecsys $A\beta_{1-40}$ CSF immunoassay, 80 to 1300 pg/mL for the Elecsys t-tau CSF immunoassay and 8 to 120 pg/mL for the Elecsys $p\text{-}tau_{181}$ 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 "UPENNBIOMK_ADNI-DIAN_ES_2017_18".

Exploratory Elecsys $A\beta_{1-42}$ CSF immunoassay measurement results above the technical limit of 1700 pg/mL have been provided by Roche Diagnostics based on an extrapolation of the calibration curve and will be identified in the .CSV file "UPENNBIOMK_ADNI-DIAN_ES_2017_18".

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.

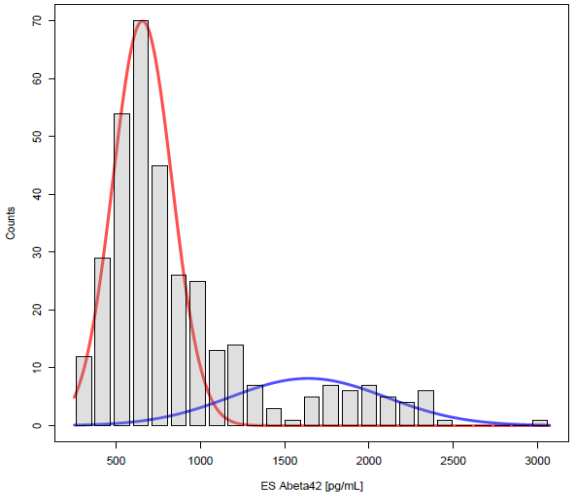
As part of the validation process for the $A\beta_{1-42}$ test method, Roche conducted collaborative studies of comparisons between the Elecsys β-Amyloid (1-42) CSF immunoassay and two reference methods (3,5,6) certified by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) (5,6).

The frequency distributions for the measurement results of the described Elecsys immunoassays, including $A\beta_{1-42}$ and the ratios including $A\beta_{1-42}/A\beta_{1-40}$, and $\log_e$ t-tau/$A\beta_{1-42}$ and p-tau/$A\beta_{1-42}$ are shown in Figure 1A, 1B, 1C and 1D for the 422 ADNI samples included in the DIAN-ADNI study.
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 determining the contribution of this and other factors on cutoff values for $A\beta_{1-42}$, t-tau, and p-tau_{181} that can be transferred between studies (4).

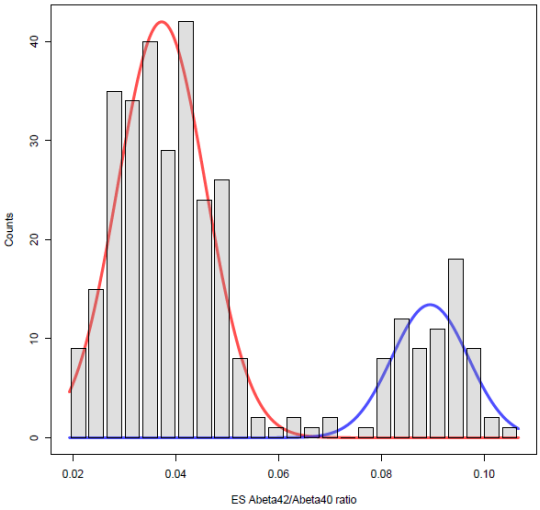
Ongoing analyses will further describe the technical and clinical performance characteristics for the Roche Elecsys immunoassays for detection of AD pathology.

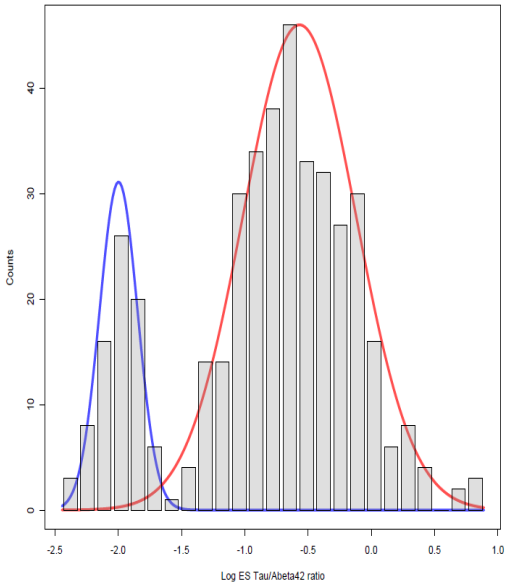
Figure 1. Frequency distribution histogram plots for **A.** CSF $A\beta_{1-42}$ alone, **B.** $A\beta_{1-42}/A\beta_{1-40}$ and **C.** and **D.** $\log_e$ of the ratios, t-tau/$A\beta_{1-42}$ and p-$tau_{181}/A\beta_{1-42}$, respectively, in the 422 CSF samples from the subset of ADNI participants included in the DIAN-ADNI study. All samples were analyzed by the Roche Elecsys immunoassays on a **cobas e** 601 instrument as described above. 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(7,8,9). 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.

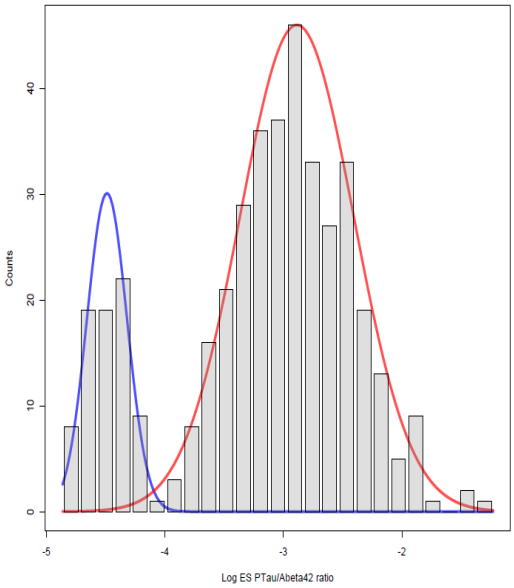
A. Frequency plot for CSF Aβ42, pg/mL



B. Frequency plot for CSF Aβ42/Aβ40 ratios







C. Frequency plot for $\log_e$ t-tau/Aβ42

D. Frequency plot for $\log_e$ p-tau181/ Aβ42

Figure 2. Precision performance for a CSF AD-like pool for the 14 days of runs for the ADNI and DIAN CSF analyses for Aβ42, Aβ40, t-tau and p-tau measured with the Roche Elecsys immunoassays on a cobas e 601 instrument.

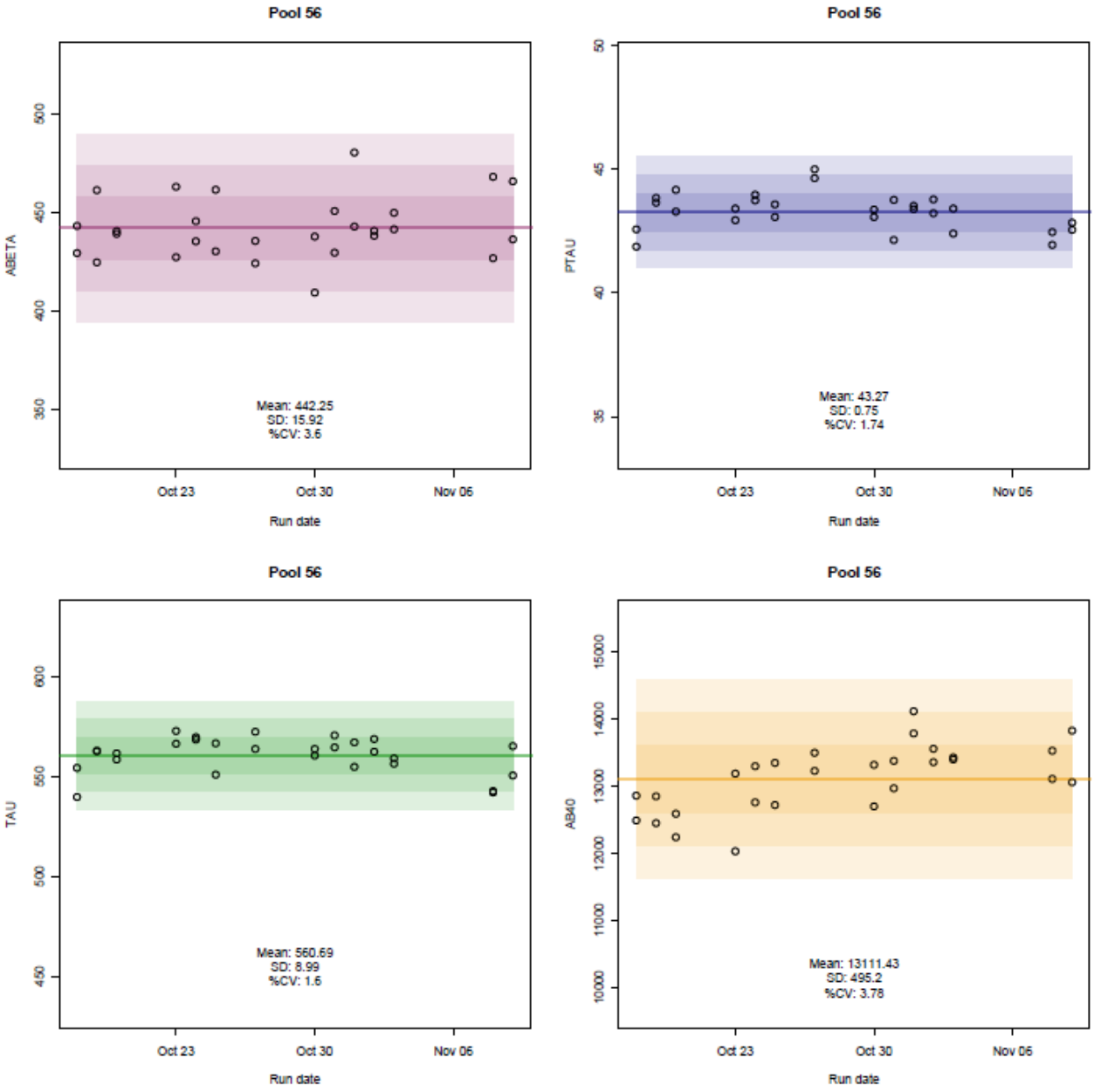
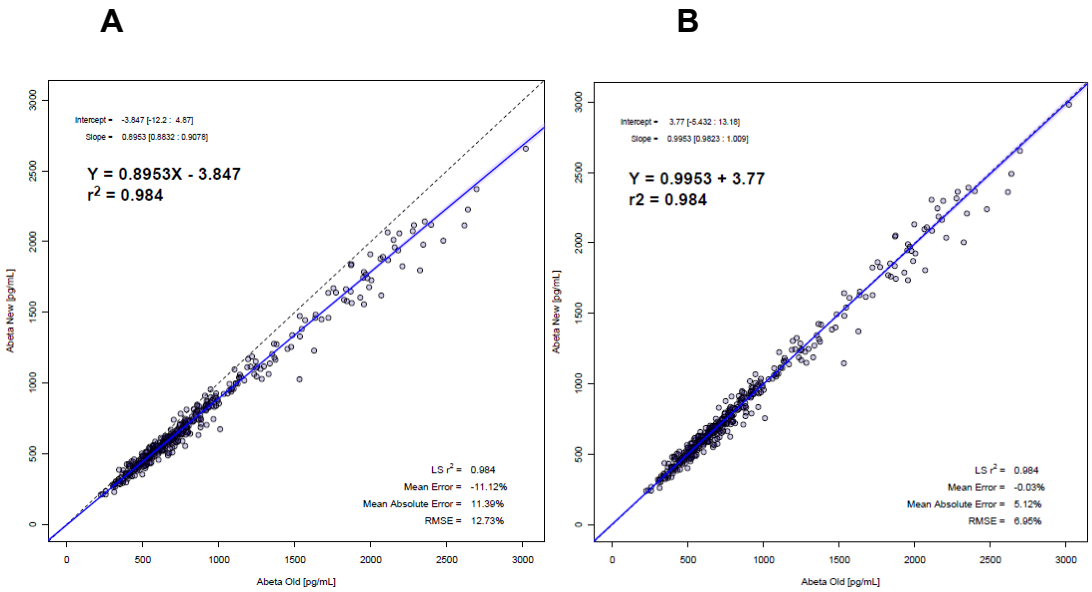


Figure 3. Test-re-test for the 422 ADNI CSF pristine aliquots that were run as part of the DIAN-ADNI study. A. linear regression plot of Aβ42 concentration for 422 pristine ADNI CSF aliquots analyzed as part of the DIAN-ADNI study vs Aβ42 concentration for replicate pristine CSF pristine aliquots analyzed earlier as part of the ADNI1/GO/2 dataset (datafile: "UPENNBIOMK9" .CSV file on LONI). B. linear regression plot of Aβ42 concentration after correction of the concentrations of the 422 concentration results



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