

**Global Biomarkers Standardization Consortium
In-person Meeting at AAIC
Saturday, July 13, 2019
5:30 – 8:30 p.m. Pacific Time
Intercontinental Downtown
Los Angeles, CA
Summary**

Re-standardization of the A β 42 assays vs CRMs (Round Robin) – Britta Brix

- Euroimmun, Fujirebio and Roche participated in the Round Robin with the objective to investigate consistency between the CRM-adjusted concentrations measured in pooled CSF samples using the different A β 1-42 company assays. The conclusion was that re-standardization was feasible and the bias between individual vendor assays are minimal and committed to commercialized readjusted tests.
- Finalized recalibration of tests: for conversion factors, Fujirebio average test recovery of serum was 146% and calculated a conversion factor of 0.46 on master calibration curve, Euroimmun conversion factor of 0.3 and Roche's conversion factor was off 27%.
- After re-standardization using the BioFINDER study data the cut-off values referring to PET positivity were still high. Fujirebio and Euroimmun decided to conduct a small study where they compared 25 frozen, neat CSF samples in re-calibrated tests. Both assays still measured CRMs in their targeted ranges. The correlation was good in neat CSF samples; however, the samples are behaving slightly different from CRMs and despite recalibration still difference between values of 21%.
- Plan to have a Round Robin 2 to address sampling handling issues in September 2019.
- Questions the team had were: What is the difference that is acceptable in the field? Do they need to improve? Can they improve with the methods they have? What is the global cut point? Can they have a reference cohort that can confirm that they have met the target value in this cohort that can be used for cut point redeterminations? All work together to streamline future serums so future re-adjustments are done at the same time?
- There might be assay differences affecting data but an additional Round Robin is necessary first.
- The group discussed whether the calibrators were the same within the companies. The problem may not be the analyte but the differing matrices the companies use.
- What is the lot-to-lot variability?

Update on QC Program / NFL – Kaj Blennow

- Total CV between labs is 15.3%, there is minimum batch-to-batch variability.
- Assays included Innotech ELISA-A β 42, A β 40, tau, p-tau, same analytes with Euroimmun, IBL, Lumnipulse and Elecsys, Mesoscale - A β 42, A β 40. Have added NFL in the last year.
- Means for A β 42 concentration are: Innotech is 13.8, Euroimmun is 7.6, Mesoscale 19%, IBL-8, Elecsys-6.6, and Lumnipulse is 11.7.
- A β 40- IBL is 12, Elecsys- is 3 and Lumnipulse is 8.5.
- t-tau- there was a difference between automated instruments and ELISA methods. Tau has better CVs than A β peptides.
- They have conducted a first round for NFL with 9 labs measuring CSF using human diagnostic ELISA, there were high CVs.
- Have sent out requests to labs to incorporate plasma samples for A β 42, A β 40, tau and NFL and plan to start this next year. Asked members to participate in QC program with plasma.

NFL Multicenter Assay – Charlotte Teunissen

- Started by the Multiple Sclerosis Society, goal to evaluate precision, sensitivity, parallelism and consistency of serum sample measurements with the Simoa NF-light assay.
- Experiments began in September 2018 and 17 centers performed 6 kit runs on individual days following a standardized protocol and material measurements.
- Amsterdam, Basel, Dresden, Ottawa Quanterix, Sanofi site data was reported, the calibration curves were aligned, there was a limit of detection over 5 runs with the mean below 0.10., lower limit of quantification is 0.2. For consistency/precision, there was a good alignment.
- Progressive MS Alliance Fluid Biomarkers Implementation Planning Team: the problem is the need for better biomarkers for Progressive MS at individual and group levels: prognosis and treatment response. Align with Alliance goal to develop smaller, shorter trials.
- Fluid Biomarker Implementation Planning Team: The primary goal is to develop and validate a serum biomarker; the team is focusing on serum NFL.
- Highest priority Context of Use: 1. As a prognostic biomarker to be used for trial enrichment that can predict disease progression in MS; 2. As a treatment response biomarker to be used as a possible endpoint in clinical trial in Progressive MS.
- sNFL in Progressive MS- the gaps in knowledge are to define standard procedures in order to support analytical validity, establish a reference database, conduct a deeper analysis of legacy datasets
- Suggestion was to focus on relapsing remitting MS as well. Many drug companies use NFL as an outcome measure in MS.

Reference Materials – NFL – Kaj Blennow

- Develop reference material for NFL in blood.

- A Commutability study is needed to verify that different analytical methods correlate, and at the same time to identify a candidate reference material. The workgroup plans to work on selecting one or more Reference Measurement Procedures (RMP), or reference methods, i.e. gold standard methods, as well as a Certified Reference Material that will have a value assigned by the RMPs. The standardization procedure also involves the identification, characterization and integration of a master collaborator.
- A workgroup was formed that that will perform a commutability study for 40 plasma and 40 sera, individual samples. They will measure individual samples with several assays (preliminary including three different versions of Simoa, MagQu, MSD, Elecsys and Olink), including seven different methods.
- For candidate CRM will test buffered plasma, serum, artificial plasma and buffer, that also will be spiked in with different types of spikes of purified bovine and recombinant NFL, as well as CSF (that contains much higher NFL levels than plasma).
- It was suggested to also translate to CSF, if this is to be completed the group would need 3-4 liters of CSF.
- If labs want to participate but do not have funding the Alzheimer's Association can have a conversation with the lab to discuss possibilities.
- The members wanted to know if the WG tested the stability of the analyte in serum vs plasma. It was stable in serum at different conditions; one was RT, 4°C and -80°C but can be less stable in plasma.
- There was an inquiry on whether they were measured NFL was full length or truncated? The exact nature of serum/plasma NFL is currently uncertain.

Update on the Round Robin for Plasma A-beta – Henrik Zetterberg

- Optimized method for plasma A β by immunoprecipitation.-MS/MS and levels of A β 42 and A β 40 in plasma can be measured in 0.25ml of plasma with high precision.
- Insight46 cohort data showed high concordance of plasma A β 42, A β 40/A β 42 by IP-MS/MS and brain amyloidosis.
- Goal of the Round Robin was to examine how methods for plasma A β 42, A β 40, A β 42/40 and APP 669-711 compare: correlation coefficients, absolute values and whether correlations are linear not to evaluate clinical performance.
- 81 individual samples selected based on CSF A β 42 prepared with identical pre-analytical methods.
- Elecsys vs MS and Elecsys vs. Simoa or ELISA: A β 42 results showed moderate correlations.
- MS methods showed correlations but not ideal level.
- A β 40 correlations are better in -between most methods.
- The matrix is the challenge, methods work for CSF but a challenge when moved to a plasma matrix.

- Conclusions were there are acceptable correlations for plasma A β 40 using different methods; poor correlations for plasma A β 42 using different methods, the methods correlate considering the CSF results.
- Potential explanations were different methods measure different pools of A β 42 but still show diagnostic utility; different methods are sensitive to pre-analytical sample handling; plasma A β 42 concentrations are still at a lower limit of quantification.
- Important variable that should be considered is if there is a way to design the study that at the end it can be determined what context these assays perform for their intended use. Can use different clinical sets/stages of disease across the assays to get a better sense of property of assays for their intended use. Ex. for screening assays, assays potentially behave differently based on stages of clinical disease.
- FNIIH is developing a study that consists of comparing 6 of the different platforms and validating a cohort of 500 samples focused on presymptomatic A β positive.

A β 1-40 certification project - Sébastien Boulo

- A β 40 alone is not a biomarker with a high clinical utility
- A β 42/ A β 40 ratio seems to improve the diagnostic
- For the CRM development, a round robin study will be launched using mass spectrometry measuring A β 40 in CSF. About 30 individual samples will be analyzed and laboratories will use their own methods and instrumentation. However, the same calibrant (provided by JRC) will be used. Also, immunoassay measurements may be added in order to evaluate the correlation with the mass spectrometric methods. Furthermore, pooled CSF will be spiked with synthetic A β 40 peptide to assess the possibility of spiking.
- If certification occurs into existing CRMs (ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC), processing, homogeneity and short- and long-term stability studies were already performed.
- A calibrant for the calibration of LC-MS measurements for the value assignment of the CRM is being characterized. Two batches (HFIP and TFA) were produced and the concentration determination is done by amino acid analysis (AAA). Results from AAA show higher variability between amino acids for A β 40 HFIP. A β 40 TFA will be kept for characterization.
- For the CRM characterization 6 laboratories or methods should be used (LC-MS validated). Three samples measured in replicates over at least 2 days and a common protocol for calibration will be used.

Standardization of Alzheimer's Blood Biomarkers (SABB) Workgroup Update - Charlotte Teunissen

- The strategy of the WG was to gather inventory of standard operating procedures and establish variation, vote for most important factors to be studied to fill the gaps and establish a central repository of mistreated samples to address the gaps; define preanalytical factors and generate unified SOPs with 1 for research and 1 for clinical practice.

- Some of the preanalytical factors that were surveyed, and inventory results were shown. These included processing issues: time from collection to centrifuge, temperature during period from collection, storage issues, etc.
- There were 16 responders; there was variation in time from collection to centrifugation and variation from centrifugation to freezing, anticoagulant concentration, the temperature during various periods. The aliquot size differed and collection protocols had a huge variation. The needle size and location of draw did not vary neither tube collection order.
- Priority voting was for anticoagulant type and concentration, tube type and additives, time from collection to centrifugation and time from centrifugation to freeze.
- The WG will address the top 12 variables.
- Next steps are to establish a central repository of mistreated samples. Focus on the top 12 identified pre-analytical variables and focus will be on amyloid, NFL, p-tau, t-tau. Establish the repository in a few months, the IRB approval almost reached. Next, the WG will coordinate analysis and they aim to collect the datasets before the end of the year.
- Participation is welcomed, if interested email Charlotte Teunissen, c.teunissen@vumc.nl and Rebecca Edelmayer, rmedelmayer@alz.org
- There was question on the effect of proteases on the plasma samples?
- MIRIADE international training network. Marie Curie program. The aim of the program is to train a novel generation of scientists to accelerate dementia biomarker development. 15 PhD students will be hired: if interested email MIRIADE@vumc.nl