

Global Biomarkers Standardization Consortium
Minutes of meeting – July 12, 2014
Copenhagen, Denmark

- I. Welcome and introduction – Maria Carrillo
Maria reminded the group that the GBSC is an open consortium with no membership required.
This group has generated a lot of excitement due to its work with the IRMM (Institute for Reference Materials and Measurements) project (see section V). Although we have not achieved standardization per se, the field is moving towards using CSF measures in a more standardized way.
Maria introduced Jim Hendrix of the Alzheimer's Association, who will be working with GBSC, the Research Roundtable and other academic/industry partnerships.

- II. Alzheimer's Association QC Program – Ulf Andreasson
 - a. QC program started in 2009; now has 100 labs in database.
 - b. 3 samples (2 unique, 1 longitudinal) are sent to participating labs 3 times per year.
 - c. 4 assay platforms from 3 companies are used (Fujirebio, MesoScaleDiscovery [MSD], Roche)
 - d. Project is coordinated in Mölndal at the University of Gothenberg/Salhgrenska Academy.
 - e. Goal is to reduce variability across labs, facilitate improvements in existing assays and the development of new assays.
 - f. Commutability testing of correlation for 5 different commercial assays and an in-house SRM is good, but there is a need to show that pooled CSF behaves like individual samples.
 - g. Commutability testing of different assays/manufacturers is incomplete; will send out when we have data from all 21 labs.
 - h. Comparison of different methods used at 21 labs show good correlations but give different results, demonstrating the need for certified reference material.

- III. Reference Method Certification – Josef Pannee
 - a. JCTLM (Joint Committee for Traceability in Laboratory Medicine) submission for A β ₄₂. JCTLM is a cooperative
 - b. ISO document defines Reference Measurement Procedure (RMP) as a “thoroughly investigated measurement procedure shown to yield values having an uncertainty of measurement commensurate with its intended use, especially in assessing the trueness of other measurement procedures for the same quantity and in characterizing reference materials.”
 - c. Requires validation of the linearity and range of calibration, specificity, accuracy, precision, detection and quantification limit, recovery of spike, robustness (stability of sample and analytic procedures), and expanded uncertainty of measurement.
 - d. Submitted paper on MS-based candidate reference measurement procedure, which was accepted for publication. After paper was accepted (Clin Chem 2014. 60:7:

987-994), sent in nomination to JCTLM. Hope to get a response by the end of January.

IV. CSF Round Robin Program – Les Shaw

- a. Focus on measurement of CSF A β ₁₋₄₂ with srm/tandem mass spectrometry; using 3 different mass spectrometry systems, 3 different HPLC systems, 4 different calibration matrices, single-plex, tri-plex, or penta-plex methods.
- b. Four participating labs -- University of Pennsylvania (Penn), PPD, University of Gothenberg (UGot), Waters & Pfizer – all follow agreed on protocol to test 12 CSF pools prepared and shipped by UGot.
- c. Each lab collects data on precision/performance, recovery of spiked human CSF, freedom from ion suppression, and equivalence between surrogate matrix. Data shared among labs.
- d. Results: mean within center 4.8% CV; mean between center 12.2% CV
- e. Due to increased sensitivity of methodology, we are all down to at least 100 μ l of sample.
- f. Given the differences across the four centers, we see good agreement consistent with the ruggedness of the approach; supports working on the IFCC reference method assignment of accurate A β ₁₋₄₂ concentrations to develop CSF-based standard reference material.
- g. Issues that have been addressed: CSF stability, calibrator matrix comparisons
- h. A planned two center (UGot and UPenn) pilot study using the IRMM-preparation of abeta1-42, with mass assigned by amino acid analysis was completed that included 10 CSF pools provided by UPenn. Each center used their recently published respective candidate reference mass spectrometry methods (UPenn-Korecka et al, JAD 2014; 41:441-451;UGot-Leinenbach et al, Clin Chem 2014;60:987-994. Results: r²=0.989;Y=1.021•X – 11.8, showing the comparability of these 2 candidate reference methods. .
- i. Next steps:

Use the IRMM preparation of A β ₁₋₄₂

1. weight based vs. volumetric based dilution approach
2. Will conduct a full guided “ring trial” with 32 patient CSFs and a set of neat and spiked CSFs; hope to have two or more qualified methods for assignment of concentration to CSF pools for creation of reference methods.

Applications:

1. Compared to an immunoassay (AlzBio3) in 41 autopsy-proven cases and living age and gender matched controls showed equivalent ROC curves.
2. Provide an anchor in methods comparisons
3. Studies in various patient populations of A β ₁₋₄₂ and other metabolites for assessment of age and/or disease related changes in metabolism.

V. IRMM/IFCC Project Overview – Henrik Zetterberg

- a. Goal is to produce reference material for A β ₁₋₄₂ and have it approved before the end of 2014. To do this, we need reference methods. IFCC has a formal approach to do this. Working in collaboration with other research consortia including BIOMARKAPD in Europe. QC program (section II above) monitors and evaluates progress.
- b. As mentioned in section III above, candidate method has been submitted to JCTLM.
- c. While waiting for approval, will focus on creating candidate reference methods for total tau and phospho-tau and continue the work on human CSF-based reference material that is under evaluation by IRMM.

VI. GAAIN – Arthur Toga

- a. GAAIN provides infrastructure that provides bridges to connect islets of data
- b. GAAIN addresses issues of accumulating and distributing large amounts of data to provide greater statistical power
- c. Need standards so data are comparable and can be aggregated in order to create models and examine harmonized data.
- d. Uses a federated system because 1) too much data to keep in one place, 2) respects ownership needs, data access rules, etc. of different research groups

VII. Industry partners

- a. MSD – Bob Umek
 - i. Testing includes 3 control levels using internally developed controls; also do accelerated stability testing of components followed by real-time testing at recommended storage temperatures. For V-PLEX assays, now at 30 months of shelf life.
 - ii. Tau assay – we chose a single molecule (full-length 441), which shows good correlation with mass spectrometry method.
 - iii. Tri-plex assay – validated internally with spike recovery, specificity testing against a large number of peptides.
 - iv. Cut-points – following 8-site validation study with one lot of kit, did another multisite (3-site) study using well-curated CSF individual samples and freshly made pools with all 3 development lots. Carol Gleason from BMS analyzed these data and the results are in press. Biggest problem seen is between sites. Between-sample variation is “immeasurable.” Site-to-site variability thought to be related to different practices and other equipment involved in conducting these assays (e.g., pipetting, shakers, etc.).
 - v. MSD has now introduced the MSD Sample Analysis Service.
 - vi. One challenge of testing samples for multiple biomarkers is that the abundance of proteins is very different. Interested in detecting very-low-abundance proteins. In testing service have introduced new S-PLEX assays for sensitivity, able to measure in the low femtogram per ml range using the same plates and instrumentation. Assays are robust and reproducible, with good correlation between V-PLEX and S-PLEX.

- b. Fujirebio – Manu Vandijck
 - i. Neuro INNOTEST® improvements: ready-to-use calibrators, run validation controls, harmonized kit reagents. These have improved ease of use, reduced inter-center variability of assay. Expect to see reduced variability in the QC program
 - ii. Automation – test protocols for automated processing using Dynex Technologies DS2 plate processing instrument are verified
 - iii. Taking steps towards accuracy-based assays – bringing contexts of use together in one product. (many publications cited). Feasibility study ongoing for INNOTEST AMYLOID- β_{1-42} :
 1. review of assay variables (kit components, test procedure, assay set-up)
 2. determined minimal required dilution with 16 CSF samples. At dilution of 1:4, the average variability over back calculated concentrations was 5%.
 3. No need for Tween at test set-up (tested in 8 CSF samples)
 4. Spike recovery within 20%, no impact of increasing $A\beta_{1-40}$ concentrations, and inter-run variability = $\pm 7\%$.
 5. Comparison of feasibility defined improved test procedure to Package Insert defined procedure - $R^2=.8874$
 - iv. Launch of new INNOTEST® $A\beta_{1-40}$
- c. Roche – Tobias Bittner
 - i. Diagnostic platform: cobas e 601
 - ii. AD biomarker development program – CSF $A\beta_{42}$, tTau, pTau (no multiplexing).
 - iii. Developed to enable patient selection for Gantenerumab trials; also exploring ways to develop stand-alone diagnostic assays.
 - iv. Lot-to-lot variability: Spearman's $\rho=0.997$
 - v. Comparison to LC-MS: Spearman's $\rho=0.936$
 - vi. Comparison to Innotest ELISA: Spearman's $\rho=0.922$
 - vii. Analytical performance of all assays (preliminary results for tau assays) – all show very good within-run and within-lab and between-module precision, lot-to-lot consistency, stability. pTau181 assay shows non cross-reactivity for non-phosphorylated tau.
- d. EUROIMMUN AG – Britta Brix
 - i. Since last year, have incorporated RTU reagents and lyophilized calibrators; can return results in one day.
 - ii. Small round robin study on different automation systems showed manual and automated systems are comparable although intra-assay variability improved in automated systems.
 - iii. Bias and imprecision – 3 labs (out of 8) were outliers, but with non-spiked commercial CSF the imprecision and bias are lowered.
 - iv. External and internal QC programs

- v. Inclusion of additives (not Tween) to lyophilized calibrators had minor impact and improve long-term stability
- vi. Have done “road trip” to educate labs about the need for standardization.
- vii. New tests in development: A β ₁₋₃₈ and ApoE ϵ 4

e. Quanterix – Andreas Jeromin

- i. Founded in 2007; working with many partners around the world
- ii. Simoa – measuring proteins below 1×10^{-12} M – sensitive improvement typically 100 to 1000 fold
- iii. A β ₄₂ day-to-day repeatability – ranges from 4.88-10.18% CV
- iv. Fully automated, uses smaller samples
- v. Strategic focus on CNS within 5 different areas – metabolic, neurology, inflammation, infectious disease, and signal transduction
- vi. Open to custom assay development (homebrew) – more than 30 projects completed or planned for CNS biomarkers

VIII. CAMD FDA Qualification Update – Diane Stephenson

- a. CAMD’s AD CSF biomarker team working on FDA qualification of CSF analytes (A β ₄₂, tau, p-tau) for the purpose of enriching AD clinical trials at pre-dementia stage. Currently in consultation and advice stage. FDA requires providing data to support a regulatory decision; however many clinical trials in prodromal population are ongoing and the translatability of ADNI populations to clinical population has been raised. Currently waiting for data from failed BMS gamma secretase inhibitor study.
- b. Speed will be accelerated by access to more data
- c. Problem that there are not enough CSF specimens for the needed studies – need to find better ways to pool highly characterized CSF samples to get specific cut-points. Also, must not use precious samples on assays that are not ready. Until data show reliable biomarkers that track with outcome, focus will remain on enrichment.
- d. CAMD has made progress has been made in data standardization - pooling data from 6500 subjects from AD clinical trials taught us a lot about inefficiency when data are not pooled or standardized.
- e. In the future, FDA will require that all IND submissions will require data be submitted in a standardized format – CDISC SDTM
CAMD has developed AD therapeutic area specific standards most recently version 2.0 which includes AD CSF biomarkers.
- f. AD CDISC standards are broadly available (<http://www.cdisc.org/therapeutic>) and sponsors are encouraged to employ these standards in their ongoing and future trials.
- g. Implementation of CDISC Standards will facilitate future pooling and harmonization of AD data.

IX. Concluding remarks – Maria Carrillo

When the AAQC was originally funded, we were not sure where it would go. We saw more issues than we originally thought and this required the involvement of a broader variety of stakeholders, including assay companies, CAMD, etc. This group has coalesced into a science-based patient advocacy group. We are grateful for your work on behalf of patients and families.