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Global Biomarkers Standardization Consortium (GBSC)
Methods Subgroup Teleconference Agenda
October 4, 2012
Time: 10:30 am ET/9:30 am CT/7:30 am PT/3:30 pm Sweden/Germany

CoChairs: **Holly Soares, Henrik Zetterberg, Kaj Blennow, Piotr Lewczuk**

Facilitator: Maria Carrillo, Alzheimer's Association

Attendees:

Mary Savage	Robert Umek	Andy Lockhart
Glenn Barney	Tim West	Tobias
Henrik Zetterberg	Holly Soares	Dean Hartley
Ian Pike	Bill Mela	Les Shaw
Ilana Fogelman	Kina Hog	Adam Simon
Joel Braunstein	Diane Stephenson	June Kaplow
Josef Panne	Malcolm Ward	Heather Snyder
Manu Vandijck	Emma Scofield	Malcolm Ward

1. **Discussion of progress each lab has made in the development of the 'substitute' or 'surrogate' matrix.**
   Update regarding round robin will be given at the end of this discussion.

2. **Paul Contestable –discussion of access of OCD calibrators for assays**
   This update has been tabled until the next call.

3. **Joel Braunstein/ Tim West from C2N – discussion of a new MRM CSF Tau assay (Attachment A)**
   Representatives from C2N presented some background and information regarding their MRM CSF Tau assay. C2N is a technology spin off company from Washington University St Louis (Bateman/ Holtzman). The company has focused on two sets of activities, revolving around biomarkers for neurodegeneration.

   The team shared some data of their AB assay, allowing them to detect range of AB in CSF or whatever the biological sample. For example, in a 272 sample (within and between approximately 10-15 individuals), they can detect correlation between ELISA and mass spec. They are working on a large scale validation and clinical validation at this time.

   The mass spec assay provides a smoother curve and is a more reliable measure. For AB, they performed three independent experiments using different aliquots each and run on independent days over 6 weeks and performed a total analysis. The separate experiments determined similar ng/mL concentrations of AB.

   In addition, C2N is also developing a Tau IP/MS assay. Through this assay, they are able to measure fragments of all tau isoforms. Lower limit of quantitation by this assay should be low enough for physiological concentrations of tau and they are currently doing a clinical quantification at this point.

4. **Ian Pike/ Glenn Blarney/ Malcolm Ward from Proteome science – discussion of CSF Tau assay (Attachment B)**

Representatives from Proteome Science provided an update on the CSF tau assay. They started by asking specific questions: what forms of tau need to be measured, total tau, specific phosphosites, truncation products, other PTMs. To address these issues, they have developed several MS methods for quantification of tau and site-specific phosphorylation. They have found triggering spike concentration works best in isobaric process. In their assessment of what they could see, they found three non-phosphorylated peptides (using SRM spectra). Using MS, they have detected five total non-phosphorylated peptides of tau. In addition, they have been able to compare elution of 100fmol total peptide analyzed by SRM after micro-flow rate chromatography and get alignment of several transitions for peptide titer. They found that the 75 micron column increases sensitivity and precision values.

The team continues to develop more sensitive and comprehensive methods. For example, from a 1 mL sample, they can identify several peptides and can quantitate total tau through SRM approach and current AQUA-SRM is applicable to CSF. The TMT calibrator approach (multipoint calibration approach) can create calibration curves using several reporter labeled ions; this uses the spike reference material to introduce artificially high concentration to creat trigger to ensure peptides and phosphorylated peptides are detectable for MS/MS. Their reference sample is made of phosphorylated peptides of synthetic tau. They have developed an inclusion list and use this list to ensure mass spec is working efficiently. Despite these developments, at this point, some peptides are beyond level of sensitivity.

5. **Discuss round robin for 4 laboratories that would include use of a common, agreed on Abeta1-42 standard, and internal standard, and aliquots of CSF samples**.
   Henrik and Les gave an update on the round robin for the 4 laboratories. The procedure has been circulated and approved by the 4 laboratories. Currently, they are working to collect samples and anticipate that the project will be able to stay on target of timeline. There will be ELISA (AB42) on all samples as well because as we continue to evolve methods, it will be important to make comparisons of immunoassays. Each center very interested and sensitive to the fact that this is first time there has ever been mass spec assay for AB. Comparison will be most important and will help us ensure we are all on the same page analytically.

6. **OTHER BUSINESS**
   Henrik gave update on the IFCC working group and IRMM project. The IFCC will hold a second meeting in Italy in May, 2013. At this meeting, the group will demonstrate activities to date and at this point, the group is unsure of what the IFCC expects to see as there is no defined time table. The IRMM project is on-going and they have almost completed first pilot; on next meeting, a team representative would be able to give short presentation of this test as the data is currently in the final stages.

Next meeting will be November 19 at 10:30 am eastern / 9:30 am central / 7:30 am pacific / 3:30 pm Sweden/Germany.