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Global Biomarkers Standardization Consortium

Reference Materials Teleconference MINUTES

April 05, 2012

ATTENDANCE: Christopher Spedaliere, Bob Dean, Ingo Curdt, Holly Soares, Maria Carrillo, Henrik Zetterberg, Les Shaw, Bill Mylott, Yuan Moucon, Henrik Zetterberg, Rand Jenkins, Holly Soares, Adam Simon, David Stuart, Andreas Jeronim, Hugo Vanderstichele

DOCUMENT AND MEETING MATERIAL LOCATIONS:

Hidden Link: contains all materials and dates for future meetings

http://www.alz.org/research/funding/_global_biomarker_CSF_docs.asp

Public Link: contains general information and dates for future meetings.

http://www.alz.org/research/funding/global_biomarker_CSF_materials.asp

1) The IRMM and IFCC process:

   a. Institute for Reference Materials Measurements and IFCC, International Federation for Clinical Chemistry.

   b. A funding mechanism has been created in the EU called the Joint Program for Neurodegenerative Diseases or JPND. Kaj's lab has proposed to the JPND a project plan to the IFCC which is the governing body that asks the to consider a project by the GBSC as a reference methods for certification. Ingrid Zegers from IRMM would evaluate methods and help us establish them, this group is like NIST which is the USA group that collaborates with IRMM to accept similar projects in the USA. The two groups work very well together and do not duplicate and agree on each other's adjudications .

   c. We would propose a project for the establishment of a reference material. Work will primarily be done by global consortium and partners, because we need to submit candidate reference method to IRMM and Ingrid will be attending our future teleconferences and she will be giving a presentation in the next TC explaining the process. The project plan has not been completely finished. There is a 2 page draft work plan that is very simple at this point. A revised draft has been sent to Ingrid and she is going to review it. When she gives feedback, it will be shared with others on the GBSC.

   d. By next TC, we hope to have this project ready to circulate and Ingrid would be on the call as well, she will need to let us know how extensive the characterization needs to be and how many labs need to participate and what type of long term stability will be needed.

2) Mass spect methods that Rand and Les have put together have been discussed, and the methods group created a spreadsheet with three lab processes. Part of moving forward on mass spect, is to establish the materials. Les had committed to gather the information regarding calibrator diluents for each participating lab. Kaj and Henrik will be next. Erin has not been reached out to, though her abstract shows she is using rat plasma and reported for the first time a non aqueous protein based calibrator which may be important for future calibrators. Les has updated the excel file that has each centers' methodology and then add a 2nd tab which will contain the constituents that each lab is using. Les has been using bovine serum albumin spiked by rat serum to get as close as possible to CSF in terms of immunoglobulin.

   a. Rand commented that they have spoken to Erin quite a bit in the past few years and they have now been supplementing with rat plasma to avoid non specific binding. Rand reports that original formula needs additional protein concentration and higher amount of albuin and IgG to make it more similar to human CSF. Not sure if any additional BSA should be added or rat plasma. But this is still being evaluated.

   b. One issue is original calibration diluent or artificial CSF material is to be as close as human CSF but analyte free and no lipid currently. 2nd, whether there needs to have additional albumin or plasma or lipids prior to cell extraction. Question is do we want to make artificial csf calibrators and store them in bulk for QC, and Rand's team has decided not to do this because of the need to establish stability. So make batch samples and batch QCs upon each sample instead freshly on day of analysis. This would not require establishing stability.

3) Trying to mimic human CSF, which has been attempted in immunoassay world is not simple and there is quite a lot of trial and error. This is a work in progress. Les is in favor of artificial CSF with electrolytes and glucose etc, the goal is to move to AB1-42 alone, vs measuring in presence of 38 or 40 and Les is getting the best results in terms of noise when focus on 1-42 as analyte. Consistent with recommendations from the FDA and others, we will focus on one analyte.

4) Henrik comments that it is easier to work with FDA on one analyte at a time. We can see how important it is to include 40 and 38. Published protocols on calibrator diluents have come out from their lab, and buffers have also been published but

haven't decided on one protocol. Approach of fresh calibrators is an intriguing idea because you can calibrate own lots and this would work with candidate reference material project and make it more feasible.

5) Has anyone settled on a common calibrator from a specific company? Rand thinks that PPD peptide is first choice for purity. Rand also is thinking of testing using a differently labeled ab 1-42 as a surrogate analyte and put it into the artificial CSF and human CSF and quantifying both to see if they are parallel to each other. Need to have highly characterized material to use it as an authentic surrogate reference analyte. This will be tested out by PPD to further confirm the comparability of artificial and human CSF. Labeling for nitrogen 15, C13, chemically synthesized.

6) Are these diluents able to be used later in a more global use, Les is not sure that the compatibility for this broader use is not known. Appropriate stability and utilization is a very important question. Henrik mentions that the IRMM ref methods and materials project will include a commutability test and commercial assays will be analyzed on same reference materials and then plotted and will be evident on what assays can be used using CRM. Certain commercial assays will not commute well, this will mean that they measure some other form of the analyte and may not be possible to standardized or calibrate using the material being developed. Ingrid will be able to share some information about this and show how this has been done for other serum proteins that have reached commercial assay.

7) GBSC is very unique effort, Les comments that when this type of effort goes on, there is not always a precompetitive effort. And there is little opportunity to invite companies to review proposed diluents. But this group has that capability and operates across vendors of assays and labs developing methods/materials and might be appropriate to consider at an early point when we have a desirable mass spect diluent, would be want to invite vendors to join in on that discussion? Bob Dean thinks that is important but we should be careful not to tackle too much or we won't get anything accomplished. First perhaps establishing the material and determine a substitute matrix for mass spect will end up being very useful in the immunoassay environment. Immunoassays are going to vary based on antibodies and affinities. This analyte is very complicated, has many interactions and various assays are going to measure analyte in some cases and some not.

8) Rand mentions the Guanidine HCL treatment, which could disaggregate oligomeric Abeta forms. So possible that value measured by mass spect would be different from value in other assays. Henrik was concerned about the power of method to measure interactions because of destruction of the aggregates, but found that treatment to disaggregate did not affect the measurement and still was able to

detect the analyte in their assay. Pilot test showed that low abeta in AD vs control group is lower concentration. SRM method of Henrik looks like values are 1.5X higher in AD immunoassays vs other mass spect but still AD patients are lower than controls. Slemmon work suggested that if you disrupt the abeta with Gu HCL you might lose some differentiation and Henrik says that in pilots looks like mass spect abeta does not show that. Rand says that their works shows same thing but don't know if there is a bias introduced.

9) Pilot study Henrik reported and presented at RASAD looked at discrimination of mass spect and immunoassay in patients vs controls? Les' group also has a pilot and is preliminary as is Henrik's but both used early AD patient and controls. Les feels we need the calibrator diluent issue addressed, and so recommendation is to get this under control and then proceed with a validation test.

10) Henrik mentioned that reference method will establish a CRM or reference material and this would be a material with an assigned concentration that will be useful for 5 years or more, then would need to be redone. This would be different from Rand's description of making the material fresh each time. What gets distributed for measurement through the IRMM process is going to be ready to go. Will not need to be re calibrated each time.

11) Timeline for calibrator diluent, Les suggests a few months for each lab to come to agreement on basic constituents. Rand and Les feel they can come to agreement on constituents that each would work with diluents if there will be a common reference method proposed for a few labs. A common formula should be possible. Creating a material that is close to human CSF is almost there, just need to make sure we all agree on what other proteins will be added to the material. The only question is whether to add lipids, but Rand's group will be testing this soon.

ACTION ITEMS TO TRACK:

1) Les will update the current excel file and add a 2nd tab with diluent and constituent from this discussion, so that we all know the proposed small molecules/electrolytes and glucose content and human albumin and source and lipids so that we all know what the formula is under discussion.

2) Review of IRMM project plan from Henrik on the next call.

3) Presentation by Ingrid about IRMM will be included on the next call.

4) Henrik will share slide deck from RASAD on preliminary data looking at mass spect and immunoassay. The slides will be shown also next call.

5) Also merging is discussed as a possibility. Next call will be merged and we will consider merging from then on. NEXT CALL WILL NOT BE ON APRIL 19th, THERE WILL BE A MERGED CALL ON APRIL 20th.

Upcoming TC Dates/Times:

Friday, April 20, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden

Wednesday, May 2, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden

Wednesday, May 16, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden

Tuesday, May 29, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden