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Global Biomarkers Standardization Consortium

JOINT Reference Methods & Materials Teleconference Minutes

Friday April 20, 2012

Co-Chairs: Henrik Zetterberg & Kaj Blennow

Facilitator: Maria Carrillo, Alzheimer's Association

Attendance: John Kamins Caprion, Theresa Heath BARC, Bob Dean Lilly, Jody Courtney Saladax, Maryann Ratcliffe AZ, Henrik Zetterberg Goteborg, Rand Jenkins PPD, Les Shaw Penn, Hugo Vanderstichecle, Manu Vandijck INNX, Andrew Lockhart GSK, Paul Contestable JNJ, Charlotte Teunissen Amsterdam, David Steward MSD, Bob Martone Covance, Tobias Bittner Roche, Daniel Kidd JAI, Bob Umek MSD, Adam Simon Ingrid Zegers, June kaplow Eisai

Report on Work plan and papers:

Publications on reference materials and methods have been submitted, they are currently looking for reviewers. We will make sure that all get the articles, the August issue is when it will be out.

The work plan hasn't gone out, Ingrid gave feedback on the reference methods paper and she is going to be able to comment on a work plan, then it will be ready to submit to the group on the call.

IFCC has contacted Goteborg and the workgroup they will assign will be formed, Henrik has suggested names from this consortium, but we have not heard back from them on what this will look like; they are pleased with the response. It has been suggested that Kaj Blennow will be the chair, but has not been finalized.

Les reported that on last conference call, he was tasked to add information regarding the calibrator diluents constituents, key for MRM technology. Added the tab for MRM, and yesterday circulated to the four groups the excel sheet with added tab asking for each of them to provide that detail regarding the diluent contents. There were many differences between four centers, including rat plasma vs. BSA and human serum albumen. Goal is to put this information together in the format and circulate it to the group when that is final. This would be ready for the next call.

Ingrid Zegers, Inst Reference Materials and Methods (IRMM). Part of the EC and is part of the Joint Research Centers, work under EC and support efforts of EC, including 7 institutes in 5 member states. Mission is to create reliable measurement system, all types of measurements, wide spectrum of activities from clinical to engineering/technology etc.

Reference materials for calibration and quality control, clinical area worked on enzymes and other proteins CRP and other microorganisms. Collaborate with partners like International Federation of Clinical Chemistry IFCC, which is an agency that collaborates globally that deal with measurement problems in matrices, and work with workgroups on measurements of plasma protein like IgG, and other markers. IRMM works with the IVD industry also since they know their systems best. Good collaboration with industry also, and others. JPCTM is an international organization that approves ref methods and materials at JCTLM.

Developing reference materials is their main interest, and how they do this will be reviewed. CM need stability data to project the suitability of a reference over 10 to 20 years or more, allowing the reference materials they produced can be used for a long period of time. Efforts are needed to be certain of the values assigned are linked to a stable reference using an international system of units. Very important feature is that concentrations of peptide are traceable to a well characterized and stable reference material. Information is needed on what constitutes this material and how is it characterized. With this in place the reference material can be used to for quality control, calibration and validation, allowing the assessment of harmonization between groups. IVD materials require traceability assigned to calibrators, which is linked to a stable reference. With this stability and quality control comparable results can be obtained in different countries.

Using specific definition of units, along with using a pure protein concentration linked to a primary method that can be used to assign a very reliable value which is used to calibrate measurements and assign values to the working material. Once a working method and calibrator is developed a product calibrator can be generated. Reference material must be fit for purpose and commutable, and years of previous research can help to ensure that this group may appropriate materials and protocols in a timely fashion.

Use of a common standard will help with comparability. First requirement is that values are traceable to a stable protocol, and needs to be commutable and that they are homogenous, stable and in the correct concentration range. Feasibility studies are needed before making reference materials, and to see if some methods are more appropriate than others. Values of AD biomarkers have been discrepant, but in her view they have correlated with each other. A possible problem is that certain assays, like the Abeta peptide immune assay, recognizing a subset of a peptides, often one specific form. Details on what the assay measuring is needed to harmonize results from methods with a broad selectivity. Also a framework is needed for a concept for tracability and proposal for starting materials. The IFCC would do a feasibility study on this.

A slide was presented showing that CRM planning is very complex and provided an overview of what it takes to accomplish.

Other markers were discussed in addition to the Ab42 project, namely total tau (t-tau), and p-tau, discussion with Goteborg and Piotr Lewczuk. Previous studies suggest Ab42 as a promising reference material, but will require significant analyses. T-tau there are some methods available on the market even without reference methods. But this would require discussion on a protein for calibration and value assignment. Technical problems for t-tau analysis is that CSF from normal controls one would need to increase concentration of t-tau to have a range that can be used for calibration. For p-tau situation is more complex, especially producing a reference material.

The different methods available will have to be looked at and determine which methods will allow harmonization with a common reference. Feasibility studies need to be done, tracking stability of the reference material is needed over 10 yrs, along with commutability studies. This work needs to be done in conjunction with companies . IRMM will do studies on material including homogeneity, stability, and quality control.

Proficiency testing studies will be needed to determine if there is substantial variability between labs, which will be used for quality assessment. Value assignment will be reviewed and shelf life needs to be assessed. Finally, documentation and certification will be needed as an endpoint .

Ab42 project seem very feasible, the others markers more complicated. Ingrid mentions that this Alzheimer'sproject is very difficult, but this consortium has good people with the knowledge and expertise to make it succeed. This group has decided to focus only on Ab42 which is more defined molecule and chance of success.

To ensure stability over time and storage, IRMM will store at different temperatures (including liquid nitrogen, -80, -20, and 4 degree C) over 2-4 years and over that time will measure stability and do reference studies. At the end all samples will analyzed in one sequence and see whether the storage has impacted the concentration being measured. Every year storage conditions will be compared. Ingrid is looking for 10-20 years stability. This is a big effort and takes 2-3 years to produce initial stability data. Henrik's lab actually has some material that has been examined over the years regarding storage conditions. There is a log file they can looked at to determine if -80 storage has maintained stability.

Dan Kidd Janssen has also done this over 3-4 years but ran out of aliquots to continue. Had some changes over time but were all relatively stable for most, small decline for p-tau but not outside criteria. Manu Vandijck commented on that measurements of stability are on different batches, but have demonstrated over a 5 year period can say that acceptable correlations where observed. However, it was not clear if this means biomarker stability.

Henrik asked about long term stability issue, and if the storage data over 3 years can extrapolate to 20 years of stability? Ingrid does measurements and start stability studies right away, which requires a 2 year study before material released, and look for changes in the concentration values. If you have different groups showing stability for 3-5 years, that might be enough to start the project and IRMM will continue to do the studies over the years to ensure that reference materials are stable. They will continue to do stability over the next 20 years.

Successful of standardization of serum proteins looked at their stability over time and transfer protocols. Dilution series from standards are important, and will be up to user to create dilution materials and must be done in a centralized manner. Rand asked about three materials that could be involved from peptide standard with known amount reconstituted into artificial matrix, vs solution in artificial matrix, vs. human CSF pool. Which is IRMM interested in producing? Ingrid says they are interested in material as close as clinical sample as possible, indicating human CSF is preferred. If companies are using different matrices and methods of reconstitution, it is unlikely that the reference material will lead to harmonization. IRMM would do this in house and provide to companies the matrix material.

Henrik mentions that there is a draft work plan which we will ask for Ingrid's input and then circulate to the edited draft to the GBSC for feedback . Next week we will send that to IFCC. We are also looking to attract some additional funding for the project.

Adam, what is the biggest challenge that we are facing? Ingrid replies that there are many challenges with differences in selectivity of different methods. Only certain methods may allow for harmonization. If this is the case for some of the assays, how is this dealt with? Ingrid replies that the company methods will have to reformulated to be compatible with a workable method. Other problem is production of the calibrant and need to be characterized carefully for concentration. Development of protocols for transferring values from calibrant to working calibrant is needed. This step can introduce bias and mistakes and maybe needed to specifically look at the problem. Many hurdles in development of a reference system have been studied, for example comparing source materials, thus expediting this process.

Bob Umek says the IRMM presentation was not a surprise, and on a practical level we need to go forward. He indicated that we can't wait for this work to be done, we need to understand measurements that come from an international standard. Companies meanwhile will need to make that consistency as best as possible. Bob Dean mentions that he is impressed by IRMMs most recent efforts for standardization of analytes used for clinical practice for which no similar standardization previously existed. This set of analytes being worthy of their effort speaks volumes. And existing manufacture need to move forward to advance the science and establish commercial viability. These processes may needed to exist in parallel to make progress and achieve standardization.

Henrik mentions that discussions with Ingrid are that the generation of the pilot reference materials will be large enough to invite any company to contribute to this analysis. She said that the more companies we get to participate in the commutability study the more information we will have about the candidate reference material.

IRMM wants to team up with a few labs to do these types of analyses and will be discussed in work plan. Feedback will be appreciated when the draft is circulated.

ACTION ITEMS for May 2nd:

1) Update from Les on changes to excel spreadsheet on diluents.
2) We will have an update on the IRMM project plan and the publication by Henrik.

NEXT CALL MAY 9th at 9:30am Central