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Global Biomarkers Standardization Consortium

Reference Methods Teleconference Minutes

Friday March 16, 2012

Co-Chairs: Henrik Zetterberg & Kaj Blennow

Facilitator: Maria Carrillo, Alzheimer's Association

Attendance: Holly Soares, Les Shaw, Rien Blankenstein Amsterdam, Omar Laterza Merck, Erin Chambers Waters, Theresa Heath BARC, Johannes Streffer Janssen, June Kaplow Eisai, Bob Martone Covance, Adam Simon AJ Simon Ent, John Kamins Caprion, Punkaj Oberoi MSD, Moucon Yuan PPD, Bill Mylott PPD, Henrik Zetterberg Goteborg.

David Steward, Pankaj Oberoi, Rand Jenkins, Bob Umek,Dan Chelsky Caprion, June Kaplow, Marian Ratcliffe, Les Shaw, Daniel Kidd, Bill Mylott, Moucon Yuan, Vesna Kostanjevecki, Omnia Ismaiel, Henrik Zetterberg

Les report on a comparison of the three methods discussed, Waters, PPD and UPENN

Overview of the methods group which is linked to the materials group, one of the goals is to develop a standard methods material for abeta42 and future tau. As part of this effort of developing a standard reference method that would be CSF based, which Goteborg have worked with IFCC, is to have the method that would ultimately assign values for abeta in 2 or 3 different pools of CSF covering a range of abeta42 concentrations. Among the considerations for reference methods methodology is the MRM mass spect with the development spearheaded by Erin Chambers, as a starting point to release all forms of beta1-42 and take sample and quantify abeta142 in CSF having released it from all sources of aggregation with high concentration of GuHCL. Excel spreadsheet is overview of these methods as they are being developed at each of the centers.

Erin mentions that she and Rand have updates that result in less differences in the methods than even demonstrated on the current spreadsheet.

Matrix affects were also discussed in mass spect by Les. Calibrator diluent has been a topic, and Erin started work on rat plasma and will be updating the work as we go along. Rand at AAIC2011 used bovine serum which Penn was also using. But Penn and others have been adding rat plasma back. Yuan mentioned that at PPD they are doing that testing now. Les mentions that this has demonstrated improved performance from BSA. All are using micro columns, all using GuHCL so sample prep wise except for starting volume of CSF are

comparable. Liquid chromatographic systems do vary. Solvents are comparable with minor differences. Primary analytical column is similar for all except length. Flow rate of diluting solvent is not a huge different. API mass spectrometers are different models. Run time from 8 to 12 minutes overall. LLOQ is from 25 up to 100. Penn is now at 50. Abeta42 accuracy of calibrators has been noted. Human CSF spikes show good results with bias being close to zero. Comparable results with recovery in spike of peptide from waters, ppd, penn labs. Precision of QC samples is not as good as could be but is getting better as we optimize system. Calibrator precisions are getting better as add rat plasma to BSA.

Diluents were discussed by PPD and Penn. HSA final concentration and human IgG were discussed with updates. By doubling HSA and IgG you come closer to the actual content of plasma. The need for artificial CSF has escalated and simply using this is not a proper mimic for human CSF. Artificial CSF is either the commercial CSF or the one that is home grown.

This groups' strength is that discussion helps improve precision simply by sharing information on the diluents and other aspects.

Next steps are that this is a hot research topic, and that criteria should be that we aim for not going outside of 5-7% CV criteria. Waters and Penn agree. PPD agree as an ideal but Bill thinks this is a big challenge. Bill mentions that to use large molecule criteria of 20-25% CV and evaluate the data then set criteria, which could be as low as plus minus 15% CV. Erin and Les mention that current reports are already at 15% so Les feels that they may be able to set goal of 5-7%. Holly agrees with the lower CV goal as well. Erin mentions that their average accuracy numbers are in the single digits.

Erin mentions that the issue of being able to use this in the clinic, the variability is too high and doesn't allow you to distinguish between populations. This speaks to longer range goal of moving into clinical practice with this tool. When you think of the subtle changes that occur with this disease, it requires as much precision as possible.

Adam asks about preventive maintenance from companies. Les comments that they find it necessary to clean the system after apparent buildup of material. When cleaning is done, one cannot run an assay but run repeated samples with CSF samples as if there were hotspots being released in entire mass spect system. Others may have other ways of cleaning and tuning equipment. System changes/perturbances can wreak havoc on the assays. How do Waters and PPD handle this? PPD, Bill replies that they do find contamination and systems that need to be cleaned more frequently.

Omar mentions that this may be too much in the weeds, and propose having a discussion on how to go about assessing each method and what method should be used and how to base decision to select the reference method. Les mentions that there is a need to do an

interlab study to verify the performances that have been worked out. Oman mentions that perhaps we are going to unifying these methods to try to have them be the same and all be reference methods, or take each one as it is and pick one from them. What is the goal? Unification or pick one?

Les thinks it can be a combination of this. Each lab has method that fits their purpose, at end of exercise goal is to define one. And a few labs may qualify and wish to be involved.

Basic mass spect conditions seem to be pretty close at this time.

Harmonization requires collaboration that has occurred on this methods group. Les mentions that we may not have enough experience to pick one diluent etc. Reason is that as you make changes there need to be many runs to ensure the validity. Adam agrees with Les that there is progress with sharing in this group. Harmonization is moving forward on this group with Penn, Waters, PPD and Goteborg is making progress. At the right point, we would hopefully recognize a stable and harmonized protocol and do a study to show that it is stable enough to show us the right method.

Convergence has been ongoing and is coming together in Erin's view. Through these conversations we have made this type of progress. Holly agrees in one way with Omar that we do need to think strategically to delineate our stage gates, what are we working toward? Omar agrees, we all want to harmonize these assays and what is not clear is what we will do when we are done harmonizing these.

Henrik joined and proposes that he send out some CSF samples to all to measure and see where we are. Les is not sure if we are ready for that next step though it is an excellent next step to shoot for. An exchange of samples and preliminary pilot investigation would be needed. But calibrator diluent is still being worked out and calibrator goal is not met.

Omar asks of PPD who works in strict GLP space, is it possible to conceive of a single method on three or four different platforms? Henrik says that the platform doesn't have to be identical as long as the method is the same according to the IFCC. Omar mentions that some mass spec platforms are cleared for regulatory use and some are not. Erin replies that for this type of assay none are cleared by regulatory. Requirements for clinical use are very strict. This is not for IVD but just a reference method.

Henrik mentions the method paper that has been drafted has been sent to Ingrid at IRMM for approval. He will then send to this group so that if you want to be a co-author then you can just email him. But also you can comment and add information. By next week there should be a draft circulated.

A group will create a project for IRMM and will interact with them on this. The group will be smaller and will be formed soon.

Holly mentions that we have made tremendous advances with knowing what is going on in each of the labs. What are next steps for the calls?

Les is motivated to get together with Erin and PPD and Goteborg on diluent constituents. Les has been playing with mixing into BSA the rat serum. Ultimate diluent would be human protein based. Human serum albumin some IgG and other constituents. PPD and Penn spoke and certain concentrations were being considered. PPD and Bill's effort in defining the diluent is to try increased human serum and IgG. Rat serum could be potentially difficult. Rat plasma, edta or halperin? Edta is what Erin uses and halperin hasn't been used. Risk of using rat plasma is such, due to the variability and uses something more controlled that we create a recipe for.

Les proposes to continue discussion offline on artificial CSF which is a key item to settle on. And final solution, HSA/IgG based with other additives is very promising. Les thinks we can focus on this over the next few weeks, and on the next call we may be able to touch base and report progress.

April 6th as a touch base seems ok to Bill and then shoot for the 20th also as a more conclusive touch base. Erin feels that is unlikely she can do much that would be reportable by the 6th and try to shoot for the 20th. Converging on a human base as opposed to bovine is the topic. The target would be month for a real progress report.

Omar could lead a few people to speak offline on design and propose what the reference method mass spect criteria should be for one method. Can also discuss what parts of the assay are not flexible and must be same and which are. Adam could work with this and Rand was volunteered by Bill for this discussion. Volunteers are Omar, Adam and Rand for this discussion offline. Validation reports would be helpful from labs if they can for this discussion. Though validation reports may not be available quite yet. Maria will work with Omar to reach out and ask four groups to see if they have these available. Les feels that changes as we cooperate means that we need to redo validation so we may not be ready to have this or ask for this. Omar agrees but if there are any available then we should review them. If not, Omar agrees then we need to wait. Les agrees and thinks that for his lab some validation needs to be redone as they tweak this.

ACTION ITEMS for APRIL 6th:

1) Update from Les on update of this conversation on diluents.
2) We will have an update on the IRMM effort and publication by Henrik.
3) Omar and others will draft gold standard wish list for reference method mass spect criteria for one method. Volunteers are Omar, Adam, Erin and Rand for this discussion offline. Validation reports would be helpful if available from labs if they can for this discussion. Maria will work with Omar to reach out and ask four groups to see if they have these available.

METHOD GROUP NEXT CALL DATES:

Friday, April 6, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden
Friday, April 20, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden
Wednesday, May 9, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden Friday, May 18, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden
Wednesday, May 30, 2012 – 7:30 am PST / 9:30 am CST / 10:30 am EST / 4:30 pm Sweden