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Global Biomarkers Standardization Consortium (GBSC)

Methods Subgroup Teleconference MINUTES

Tuesday, June 26, 2012

Time: 11:00pm EST/10:00amCST/8:00am PST/5:00pm Sweden

Facilitator: Maria Carrillo, Alzheimer's Association;

ATTENDANCE: Maria Carrillo, Les Shaw, Bob Dean, Robert Bowser, Dean Hartley, Holly Soares, Erik Portelius, Bob Martone, Bob Umek, Eric Simon, Chris Spedaliere, Tobias Bittner, Punkaj Oberoi, Theresa Heath, Bill Mylott, Yuen Moucun, Kaj Blennow, Rand Jenkins, Johan Gobom, Josef Pannee, June Kaplow, Erin Chambers, Henrik Zetterberg

Les gave an update from the last call. Erin Chambers pioneered the extraction and cleanup step to permit protiolytic clean up, Erin's data is in the excel file and each lab continues to work on methods so this file will be updated continuously. Rand reviewed on the last call, regarding their efforts/PPD on this area. And then Swedish colleagues reported some technical difficulties, their review was not completed. We began conversations about matrix, which was described as CSF. And we did not go to completion with that discussion. And so our Swedish colleagues will be giving overview.

Eric reported from Sweden that they have been looking for different ways to create standard matrix. Swedish group thought to use CSF as matrix when measure the standard curve. There is a method, reverse curve method for making standard curve, which makes this possible and this is very useful. Instead of adding Abeta standard, we introduce abeta and make standard curve by ration of heavy to light abeta instead of light to heavy. Plotted conc heavy divided by conc of light. By doing this they create a standard curve and unknown variable is spiked with known amount of heavy peptide and can do the quantitation of normal. This is useful because there is no additive to the standard curve or samples and use endogenous light peptide for the quantification. This method has been described a few years ago and the initial results look promising.

Les mentioned that we look at each of the four methods there is a difference, there are good reasons for each case but this is important for creating a method description. Bob Dean

asked when they do the heavy isotope labeled standard addition, there was an observation or issue of the time/temp dependence of the signature to reach equilibrium. Eric replied that they have not studied this, but they do incubate for an hour at room temp.

Kaj also replied the question to other labs about spiking peptides into aCSF standard or samples of native CSF? Bob mentioned that they have taken EDTA plasma, serum and CSF and did spike experiments with cold and hot abeta40 and 42, and did chromatography, and found that there was a time and temp dependent signal loss in terms of immunoreacitivity but isotope was shifting to a protein bound form so saw molecular weight change. Tried to find out what time/temp dependence was. Concern is as you add heavy isotope, is it achieving same conditions as endogenous.

Kaj thinks it should be same for examining spiking heavy peptide into samples. Bob's point is do we have any data regarding adding external material, cold or unlabeled, has any one studied the time dynamics of equilibrium time. Les' lab has not done this in detail. Only for how long they equilibrate internal standard when spike into CSF pools or add in CSF standards. This is an important detail.

Rand also has not characterized this in a comprehensive way. They routinely require 30min equilibration time after spiking. But not done a profiling experiment over longer time course.

Rand has a question about reverse curve method. Rand has been doing some evals using this and using a surrogate analyte, added into normal matrix. Tried to demonstrate equivalent of artificial matrix to normal matrix. But have also used a third differentially labeled analyte as an internal standard. Rand asked how varying concentration is measured as heavy is varied. Eric replied that they use a curve on xy axis. Determine endogenous conc using the data from the standard curve. And basically take the ratio of light to heavy and multiply by the known concentration of the added heavy for each standard point on curve and then take average.

Les asked each lab to update further details on the excel file that will be sent around. Les will do a short review of their methodology. Les has been doing spiking experiments, linearity then precision of calibrator. Assessing performance of the system. Les is testing Erin's published work, building a calibration curve with hCSF, do you get same concentration that you get with surrogate matrix. Essential to validate use of surrogate matrix as performing equivalent to hCSF. Reporting this at AAIC in Vancouver.

Different mass spectrometer model is being used in each lab. What criteria would be accepted for calibrator precision, pools of CSF and their reproducibility in long run we may

want a concensus about this. Core items of interest is use of surrogate matrices and CSF matrices. Rat plasma, bovine serum albumen and hCSF based matrices, there are experiments going on to test for the equivalence between the types of matrices.

Erin joined the call, Erin thinks that more controlled matrix is needed, rat plasma is not well controlled, and among options to standardize method, the original work with rat plasma is problematic due to variability. Erin recommends that we do not use that option because of potential variability from sources of rat plasma.

Les mentioned that we are looking for reproducibility, calibrators and human CSF patient samples. Erin mentioned that she looks for regulatory guideline as less than 15-20% as lower limit of quantitation. Safer to develop assay achieving less than 10% for calibrators with exception of lowest which is less than 15%, keeping with idea of having a buffer. Being too close to regulatory limit is not ideal. So most want 7%, but Erin opines 10% for all except lowest then would be good. Kaj mentioned that this sounds realistic.

Kaj mentions that manufacturing and purity for standard peptides are important but also need to determine the concentration of the amount of the standard, and this is done by different labs for each of the four methods stated. How is this done, weighing, immunoassay and how exact is this?

Les suggests that his lab will start to document what they do, relying on peptides high purity greater than 97% and DNA determined sequence material, then weigh it analytically which is as far as they go to determine concentration. So not a primary standard. Erin follows same process.

Kaj mentions that we are working on reference method, and we need to work on reference material, but should we be starting to work on a reference calibrator. There are some discuissions with Dx companies to see if they would be willing to donate their calibrator. Bill Mylott makes a comment on reference standard for peptide and protein, theylook for purity determined by HPLC but also factor is peptide content and correction factor is what they look for. HPLC gives you purity of looking for, and peptide content gives yuou info on all other content. Peptides may have greater than 97% but content will be 60%. Each lot has different peptide content. And need that information every time you order it. So you need 2 techniques, HPLC and amino acid analysis. Vendors don't supply both, you need to ask for it and they charge you extra.

Punkaj at MSD experiences same, some lots have great purity and amino acid analysis differs from vendors. Disconnect from vendor claims, and what you get upon final reconstitution. So need to do methods on reconstitution. Vendor lots, 1/3 of lots turn out to be not useful.

Les says if we have stock standard, at what point would you be doing amino acid analysis? Punkaj asks vendor to do amino acid anal, but they then reconstitute and then send that off for amino acid analysis. Compare those two, and if there is more than 15% window is ok, but sometimes more than 40%, is quite variable.

Is there a best practice to agree on? Johan G, Sweden, bought peptide from vendors and problem was oxidation of threonine in abeta sequence. Johan says they got heavy labeled peptide and made aliquots and did amino acid analysis, used same diluent they use later and dissolve sample to be used for amino acid analysis so took vial with heavy aliquot then did hydrolysis in same vile and gave higher results of peptide compared to when analysis was made by transferring sample.

Rand asks about engaging one or more of vendors in this discussion for consideration as primary standard for all? Les mentions that peptide in Les' lab is a source and there are other candidates for sources, but vendors could be invited to the meeting in Vancouver. Punkaj mentioned that some vendors had discussions on improving their products, they are aware of the need for reproducibility. But some of this comes down to economics.

Rand mentions that it seems like it would be important to define what the minimum standard is, which would include purity, amino acid and oxidation analysis.

Rand mentions that in addition to minimum requirements from vendors, we may want to open up a conversation with them about more details in their preparation. We may want to know how they perform the amino acid analysis and purity. Perhaps we may want to invite them on a future TC. What suggested vendors should be seek out?

American Peptide, Anaspec, rPeptide, Bachem. New England Peptide, Pheonix are others but not the most common.

Rand mentions that perhaps there might be a questionnaire that we can ask them to fill out. Some proprietary information they may not be able to share. Les agrees.

Round Robin discussion. One of the target goals is to get to the stage where we can do a preliminary round robin study of labs. Les feels their lab is not quite ready for this. But important to start discussion. Kaj comments that we could start to plan for the preliminary study, then when all four labs are ready to go it can get started. Compare levels of four labs. This would include something like sending around same CSF samples to do assay and then compare levels. But with these standard discussion, can also send around free dilutions of abeta standards of abeta concentrations and include these in the round robin. This way we know that if we get up or down results in quantifying standard and CSF standard we get more clues as to the source of the differences. All agree that this would work. Kaj can supply labs with CSF samples. Les asks that for the standard, what would be recommended

matrix for standards? Would this be DMSO stock of standard? This would be up for discussion, but Henrik says that we could exchange standards, select 2 or 3 and as normally prepare them we would ship them to the other three labs. Bill Mylott mentions that if we could also send matrix, so if PPD makes matrix for round robin, and may be good to send PPD matrix and spike them with labs' own standard to see what variability would look like. This is a good start for a round robin. We will prepare this for the Vancouver meeting to discuss.

IFCC project and working group is formally approved. Have been discussing with Les what will be final composition of the group. More discussion will follow in Vancouver. One option can be mentioned, that is there is a possibility at the next IFCC meeting in 15th to 17 of November, Malaysia on the working group. But that takes time and funding. So it is up for discussion. This was suggested by IFCC. They would ask for a separate session for this group and also to meet separately to discuss.

Henrik mentions the IFCC workplan has been circulated, and has been very general. And perhaps we need to discuss some of the more specific needs.

ACTION ITEMS:

1) Vendors to contact/questionnaire needs to be created
2) Round Robin Project
3) IFCC project plan in Vancouver

1. Overview discussion of the GBSC SRM/MS/MS candidate reference method development, **Les** leads this:
    a. How best to proceed to develop and qualify the candidate reference method
    b. Transparency working together is an essential ingredient of what we are doing

2. Discussion of progress each lab has made in the development of the 'substitute' or 'surrogate' matrix. There are several "flavors" we have been testing, including the original rat plasma based surrogate matrix; a bovine serum albumin based surrogate matrix; a human serum albumin based surrogate matrix, and if I understand this correctly, a human CSF based matrix. So coming to a better understanding of our progress to date on the surrogate matrix is essential. In addition, it appears that we have some differences in philosophy regarding numbers of calibrators we are using and so worthwhile to review this point as well:
    a. Reviews of each lab's effort on development of surrogate matrix.
        i. Rat plasma based—**Erin** is discussant
        ii. BSA & HSA based—**Rand** is discussant
        iii. hCSF based—**Henrik/Erik** is discussant

iv. Analytical parameter evaluations, eg, Calibrator concentration range; replications of calibrator performance; LLOQ determination; spike recovery in human CSF samples; reproducibility for CSF pools at several concentration levels--**Les** is discussant

b. Reviews of other critical details—well summarized in the table provided by Rand and in our Excel summary file—will be discussed at follow up webinars and in Vancouver on the 14th of July at the GBSC meeting.

3. Discuss the possibility of a preliminary round robin for our 4 laboratories that would include use of a common, agreed on Abeta1-42 standard, and internal standard, and aliquots of CSF samples.

WebEx and dial-in information will be sent in a separate email, as well as in the meeting notice.