2015 C-Peptide Standardization Manufacturer Meeting
Minutes

Wednesday July 29 8:00 AM – 10:00 AM
Hyatt Regency, Atlanta, GA

Participants:

C-peptide Standardization Committee Members
Randie Little—University of Missouri
Daniel Stein—Albert Einstein College of Medicine

Committee members not present
Judith Fradkin—NIDDK
Carla Greenbaum—Benaroya Research Institute
W. Greg Miller—Virginia Commonweath University
Gary Myers—AACC
Jerry Palmer—University of Washington
Kenneth Polonsky—Washington University
Lisa Spain—NIDDK

Guests
Valerie Arends—University of Minnesota
Shawn Connolly—University of Missouri
John Eckfeldt—University of Minnesota
Kuanysh Kabytaev—University of Missouri
Danni Li—University of Minnesota
Curt Rohlfing—University of Missouri
Amy Saenger—University of Minnesota
Michael Steffes—University of Minnesota
Alexander Stoyanov—University of Missouri
Katleen Van Uytfanghe—University of Ghent

Manufacturer Representatives
Katsumi Aoyagi--Fujirebio
Corinth Auld--Mercodia
Carole Dauscher—Siemens
Holly Groth—Ortho Clin Diagnostics
David Kiaei—Siemens
Iris Kutschera--Diasorin
Danika Lewis—Ortho Clin Diagnostics
Maria-Magdalena Patru—Ortho Clin Diagnostics
Stefaan Marivoet—Tosoh Bioscience
Shanti Narayanan—Tosoh Bioscience
Hanna Ritzen--Mercodia
Kouichi Saga—Tosoh Bioscience
Masako Suzuki—Tosoh Bioscience
Carla Terry—Tosoh Bioscience
Chris Wisherd—Alpco Diagnostics

1) **Welcome and Introduction—Randie Little**
   R. Little welcomed those in attendance, those present introduced themselves. The 2014 meeting minutes were approved.

2) **C-peptide Standardization Update—Randie Little**
   - In 2002, the NIDDK organized a C-peptide standardization committee and funded an international comparison study of C-peptide assays.
   - Participating manufacturers
     - Alpco
     - DiaSorin
     - Mercodia
     - Millipore
     - Roche
     - Siemens
     - Tosoh
     - Fugirebio
     - Mercodia
   - Standardization studies
     - For the early studies, laboratories analyzed the samples and calibrators.
     - Later studies: Manufacturers analyzed the samples and calibrators, initially we performed the calculations to standardize to the reference method, later the manufacturers normalized their results.
     - Showed that standardization using patient samples with values assigned by an LC-MS reference method greatly reduces the variability among methods and laboratories.

    - Pooled serum calibrators with LC-MS assigned values can be used for method re-calibration by the manufacturer.
- C-peptide Reference Method/Laboratory Comparison
    - In order for manufacturers to re-calibrate their C-peptide assays to the reference method we must have it listed with JCTLM which requires a comparison between two reference laboratories.
    - We have published a comparison between the two laboratories in 2012 showed good correlation between them (r^2=0.9647).
    - This comparison data was used to submit the method for listing in the JCTLM database, the method is now listed with JCTLM.
    - We have made some improvements to our method at the University of Missouri, a recent comparison between the two reference laboratories now shows a better correlation (r^2=0.9921).
- Samples available
    - 7 levels of pooled sera ranging from 0 (undetectable) to 4.00 nmol/L c-peptide (Reference Method assigned values).
    - 80 single donor samples ranging from 0.23 to 5.24 nmol/L c-peptide.
    - Collection of a new set of pooled and single donor sera is planned.
- 2015-16 Goals for Reference Laboratories
    - Prepare another set of pooled and individual sera to ship as needed for re-calibration
    - Repeat Reference Laboratory comparison with previous and new set of materials to insure consistency
    - Prepare a new labeled standard as a backup (to be discussed)
    - Begin discussion with the CAP about matrix-free C-peptide proficiency testing (to be discussed)
- 2015-2016 Goals for Manufacturers: Begin the Process of re-calibration (A letter from ADA is enclosed in your packet)

Discussion:

New reference material

R. Little noted that the ADA feels that standardization of C-peptide is important. There seems to be some question as to how long the manufacturer re-calibration will take. It often happens with the next generation of the assay method, also some methods will require minor calibration adjustment while there will be more significant changes with other methods. Getting a CAP survey that uses actual serum samples is also important for monitoring the comparability of results. J. Eckfeldt asked how the reference method works, is it a tryptic digest followed by IDA? D. Stein said there is no digest, there is a purification process (SPE and 2D chromatography) prior to MS, we look at the intact molecule, there is no MS fragmentation. S. Marivoet asked about the status of the previously-discussed new international standard for C-peptide, is the reference method linked to this material? D. Stein said it has been sent out but the report has not been issued. The current reference method is calibrated using a isotope-labeled standard prepared at the New York lab, it would be good to have an unlabeled standard to further validate it. R. Little said the new standard is important, but there is still a question of how it will perform with the different assay methods. In the case of the WHO standard we have seen that there are large differences in results among method despite all manufacturers being traceable to it. We have shown that methods can be harmonized to the reference method using serum, whether the new pure material will be commutable remains to be seen. R. Little and D. Stein said we will need to see if we will need to make any adjustment to the reference method based on this new standard. S. Marivoet said the concern for manufacturers is ending up with two reference points for calibration, R. Little said we do not want that, the new material is supposed to be available by the end of the year. Once it is available we will validate the reference method against the new standard, hopefully the results will match up, if we need to make a minor adjustment we can do so prior to manufacturer re-calibration of assays. D. Stein said the value is being assigned by UV not MS, theoretically it should not be any different but in practice there may be a slight difference. D. Kiaei asked if manufacturers are being asked to re-calibrate because of the new standard or for some other reason(s) since it would seem that standardization has already been established, will there be additional re-calibrations? D. Stein said the goal is harmonization of all of the different assays, the NIH is especially interested in this due to the importance of C-peptide in ongoing clinical trials, the new standard is part of the effort to do this. R. Little said no one has actually re-calibrated their assays yet. When the standardization effort began we did not have a commutable pure standard; we used the best that we had available which was the labeled standard from the NY lab to calibrate the reference method. The reference method was then used to assign values to serum samples that could be used by manufacturers to calibrate their assays. We then performed several studies that showed this was effective in harmonizing the different methods. At some point, because of the new reference material we may have to adjust

the reference method. We do not know yet, but it will take time for manufacturers to actually re-calibrate. With HbA1c we had the problem of not having a true reference method or material; we nonetheless were able to harmonize the assays. Then a new reference method and material came along and the results did not match. At this point we are asking manufacturers to begin the process of re-calibration bearing in mind that the reference values may change slightly, we will hopefully know for sure very soon. J. Eckfeldt said the new pure material will likely have commutability issues. It is likely that you will still need to establish a traceability chain where you use it to calibrate the reference method then use the reference method to assign values to commutable serum samples for manufacturers to calibrate their assays. R. Little and D. Stein agreed. D. Stein further noted that the MS reference method is free of matrix effects as any potential interferences are removed during the sample preparation process.

Re-calibration of assays

S. Narayanan said the point of this process is to harmonize the results, manufacturers can do this now and if the new standard results in values changing slightly it is just a matter of adjusting the values. D. Kiaei said we do not want a re-calibration in a short time, it sounds like we are only a few months away from having the new standard. Having the MS reference method that matches the new standard would be ideal. R. Little agreed but noted the SRM is also important, that is what will translate the reference values into the assays. There are a lot of steps in the re-calibration process, some of them take time and we do not want to wait for each step to be completed prior to starting the next step. S. Narayanan said C-peptide is considered an exempt assay by the FDA so in the US manufacturers are not required to re-submit the entire assay, just the new calibration. Therefore the timeframe is pretty short, typically ~30-60 days. H. Ritzen asked if there are specific instructions as to how the re-calibration process is to be performed by manufacturers. R. Little said that is an internal process for manufacturers, we will provide the SRMs representing a range of values to enable re-calibration but it is up to the individual manufacturers to determine how they will do it. J. Eckfeldt asked if the SRMs are pools, sometimes you might get a sample that is not commutable for whatever reason so you need to check the individual samples for commutability. R. Little said they are pools, we do test the individual samples but we cannot do this prior to pooling, we can test them after the fact and discard any pools that have a problem sample included. D. Kiaei asked if manufacturers could send samples to the NY or University of MO lab for value assignment. R. Little and D. Stein said we have not done so up until now but could probably do so for a fee if it is helpful to manufacturers. S. Narayanan said that Tosoh is coming out with a new C-peptide assay; it is not re-calibrated yet. Should they test it with both the old and new calibrations? R. Little said that a new batch of serum pools will be prepared soon, hopefully we will be able to have the new standard by then and if needed incorporate any changes into the value assignments. At that point there would be no need for manufacturers to go back and look at the old calibration.

3) Clinical Update on Diabetes—Daniel Stein

- Main points
  - > 1.4 million with Type 1 diabetes (T1D) in the US; incidence rates rising
  - Type 1 diabetes is (usually) an autoimmune disease.
  - Adolescent (obese) Type 2 Diabetes rapidly increasing.
  - DM1 is a predictable disease with different phases.
  - Preventing future, maintaining and/or restoring beta cell function is the goal.
  - C-peptide is the most accurate biomarker of beta cell area and function in beta cell depleted diabetes.
  - Insulin resistance is associated with many "metabolic" diseases including obesity, hyperlipidemia, CVD, cancer
  - Insulin is often used as a surrogate marker of insulin resistance
- Natural History of Type 1 Diabetes
  - Genetic predisposition
  - Insulitus/beta cell injury in response to a putative environmental trigger
  - Over the years can lose ~90% of beta cells before developing glucose intolerance.
  - Continue to lose beta cell mass over time.
- Pro-insulin is synthesized in the pancreatic beta cells
  - Packaged into granules and cleaved to insulin and C-peptide for storage.
  - Insulin and C-peptide are secreted in a 1:1 molar ratio.
  - Insulin (but not C-peptide) is cleared by the liver
  - C-peptide is the best marker of insulin secretion
- Why preserve beta cell function? Among subjects in the DCCT intensive group:
  - Prevents short-term complications (hypoglycemia)

- Prevents long term complications (retinopathy, nephropathy, neuropathy, etc.)

- Strategies and Goals for Prevention of T1D
  - Major goal: Prevent T1D before it starts
  - Settling for slowing progression: 5 year delay could make an enormous impact on the challenge of adolescence.
  - Pre-diabetes, new-onset diabetes, versus established diabetes: More dangerous treatments easier to justify once diabetes established.
- Cure Equivalent for Type 1 Diabetes
  - Prevent onset by blocking autoimmunity
  - If T1D established, restore beta cell deficiency with transplantation or regeneration and block autoimmunity
  - Challenges of Islet Transplantation
    1) Supply of insulin-producing cells
    2) Protection from transplant rejection and autoimmunity
- Which tests to measure beta cell function in clinical trials?
  - Glucagon Stimulation Test (GST) or Mixed-meal Tolerance Test (MMTT): MMTT is more physiological and better stimulates C-peptide (Greenbaum et. al 2008).
  - Standard for most clinical trials looking at T1D is MMTT, integrate C-peptide concentration (area under the curve) over two hours
- Urine C-peptide creatinine ratio (UCPCR) is a noninvasive alternative to the mixed-meal tolerance test in children and adults with T1D (Besser RE et al., Diab Care. 2011 Mar;34(3):607-9).
  - Patients with T1D (n=51; 0.2 – 66 yr post diagnosis)
  - Fasting void; MMTT; p2hr MMTT void
  - Serum C-pep at 0, 90, 120 min; cutoff 0.2 nM as diagnostic for T1D
  - 90 min MMTT C-Peptide correlation with 2hr UCPCR (R=0.87)
  - 2hr UCPCR 90 min vs. MMTT C-peptide to detect stimulated C-peptide < 0.2 nM (95% sens, 100% specific)
  - Correlated highly with home pp 120min UCPCR (R=0.8)
- Discriminating Type 1 from Type 2 Insulin Requiring Diabetes (E. J. Besser, A. G. Jones et al Diabet Med. 2012 29:1279-84)
  - MMTT with and without insulin
  - DM1 (56), DM2 (35): all on insulin Rx
  - Results:
    1) 20% reduction peak serum C-peptide w/ insulin, but NO change in cut off for sig endogenous insulin secretion
    2) Fasting sCP α 90 min MMTT (R= 0.97)
    3) Fasting sCP ≥ 0.07 nmol/L (.21 ng/ml): 100% sensitivity, 97% specificity for signficant endog insulin secretion (90 min CP ≥ 0.2 nmol/L)
- Discriminating Pediatric Type 1 from MODY and Type 2 Diabetes (Besser, Shields et al Pediatr Diab 2013, 14:181-8)
  - Subjects

	T1DM	**MODY**	**Ped DM2**
Age onset	0-18	0-18	≥ puberty
Genetics	polygenic HLA +	monogenic HNFs, PDX	polygenic
body type	Lean	Lean	lean/obese
Antibodies	yes	no	no
DKA	yes	no	no
UCPCR	.05	3.51	4.01(nmol/mmol)

  - UCPCR ROC cut offs ≥ 0.7 nmol/mmol
  - 100% sensitive, 97% specific discriminating T1DM from non T1DM (MODY or T2DM)
- Variable Rates of Beta Cell Killing
  - In pre-T1D beta cell destruction can take years.
  - Transplant of pancreas between identical twins (with and without T1D) – beta cells killed within weeks. What is the role of memory cells?

- In pancreases of people with T1D for over 50 years, beta cells are virtually always present.
- Number of beta cells is correlated with level of C-peptide.

- Demonstration of Islet Cell function in patients with 50 yrs or longer of Diabetes (Keenan, Berger, Sun, Eisenbarth, Doria, King ADA abstract, 2014 San Francisco, CA)
  - N=211 subjects
  - Age 67 ± 7
  - A1c 7.1% ± 1.3
  - Random C-peptide; in house RIA
  - Results
    1) 24% C-peptide ≥0.3 ng/ml (100pmol/L)
    2) NO difference prevalence complications > 0.3 ng/ml vs < 0.3 ng/ml
- Faustman and colleagues measured C-peptide levels in patients with T1D using a new ultrasensitive immunoassay developed by Mercodia (Wang, C et al. Diab Care 2012;36:599-604).
  - Serum C-peptide levels measured in human subjects (n = 182) by ultrasensitive assay (lower detection limit 1.5 pmol/L), as was β-cell functioning
  - Results showed that a significant number of subjects that had no detectable C-peptide with the standard assay had measurable C-peptide with the ultrasensitive assay.
- Oram et. al. Most People With Long-Duration Type 1 Diabetes in a Large Population-Based Study Are Insulin Microsecretors (Diabetes Care, Volume 38: 323-328 February, 2015)
  - Study Design and Methods
    1) 924 patients were recruited from primary and secondary care in two U.K. centers
    2) Patients had a clinical diagnosis of T1D, were under 30 years of age when they received a diagnosis, and had a diabetes duration of >5 years
    3) Median age at diagnosis was 11 years (6-17)
    4) Duration of diabetes was 19 years (11-27)
    5) All provided a home postmeal UCPCR, measured using a Roche electrochemiluminescence assay
  - Results
    1) 80% of patients had detectable endogenous C-peptide levels
    2) Most patients had historically very low undetectable levels
    3) 8% of patients had a UCPCR ≥0.2 nmol/mmol, equivalent to serum levels associated with reduced complications and hypoglycemia
    4) Absolute UCPCR levels fell with duration of disease
    5) Age at diagnosis and duration of disease were independent predictors of C-peptide level in multivariate modeling
- Conclusions
  - C-peptide production persists for decades after disease onset and remains functionally responsive
  - Patients with advanced disease may benefit from interventions to preserve β-cell function or to prevent complications
- Insulin as a marker of insulin resistance
  - Peripheral insulin acts upon muscle.
  - Muscle is the largest insulin-responsive organ in the body, drives measurements of insulin resistance.
  - Like C-peptide assays insulin assays currently vary up to 100-200%.
  - Ways to use insulin as a biomarker
    1) Measurement of insulin and glucose
    2) Various ways of calculating response (HOMA, QUICKI, others)
- 20/(fasting C-peptide x fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report (Ohkura, Shiochi et al Cardiovasc Diabetol (2013) 12:21-29)
  - Fasting C-peptide and glucose measured in T2DM
  - Insulin sensitivity measured by hyperinsulinemic euglycemic clamp
  - Results: Glucose Infusion Rate strongly correlated with 20/(f-CPxf-PG), r=0.83 compared with HOMA-IR (r=-0.74), ISI (r=0.66)
  - Limitations:
    1) Normal renal function
    2) Initial group 15 expanded to 25 for ADA 2014

- Unresolved Questions
  - What is the reproducibility of ultrasensitive C-peptide assays in the same T1D individual over time?
  - Are very low levels of C-peptide (endogenous beta cell function) biologically significant?
  - Are very low levels of C-peptide (endogenous beta cell function) CLINICALLY significant?
  - E.g. does this translate to lower rates of complications (hypoglycemia; improved glycemic control)
  - Does this translate into a positive susceptibility for beta cell regeneration therapies?
  - Well designed outcomes of prospective trials of beta cell function using standardized testing procedures, and adjusting for multiple clinical and demographic variables are necessary.

Discussion:

Ultra-sensitive assays

M.Patru asked if there is a need to measure urine C-peptide now that ultra-sensitive C-peptide assays are available. D. Stein said that the advantage of urine is there is a lot of it, thus it can be concentrated in order to obtain very high sensitivity. Also it is more convenient for patients and is non-invasive. Whether it is better is a question, the data suggest it is as good as serum C-peptide. M. Patru asked how urine C-peptide assays would fit into the standardization scheme since the assays will be standardized using pooled serum. R. Little said it should not make a difference if a serum SRM is used to standardize assays, they should still be able to measure urine C-peptide as well. D. Stein noted that some assays cannot be used to measure urine C-peptide while others can. Our efforts right now are focused on serum C-peptide, but urine is something we should keep in mind going forward. R. Little agreed. A. Saenger asked how ultra-sensitive is defined, and how low do we need to go? D. Stein said prior generation assays typically went down to 15-30 pmol/L, the newest generation of ultra-sensitive assays can go 10 fold below these levels. A. Saenger asked if there is a defined acceptable lower limit. D. Stein said the ultra-sensitive assays are telling us that even patients with long-term T1 diabetes have a small amount of surviving beta cells, there is a lot of interest in this in terms of seeing whether this is clinically/biologically meaningful. Routine assays are useful in terms of distinguishing T1 from T2 diabetes. This is important clinically as patients with T1 are at great risk for developing DKA; these patients will always require insulin. However, a T2 or MODY patient may develop hyperglycemia but not to the extent of developing DKA. The very low levels of C-peptide are at the cutting edge, it is unclear if they are clinically meaningful at this point. We do know that subjects with levels >0.2nmol/L are at lower risk for complications and hypoglycemia, this is clinically useful. R. Little added that our goal at this point is to get the assays harmonized, we are not at the point of evaluating interferences, performance at very low levels, etc.

Proficiency testing samples

S. Narayanan asked if the new C-peptide standard is pure 33-63 C-peptide, different antibodies measure different parts of the molecule and some cross-react with proinsulin. D. Stein said some assays do show cross-reactivity with proinsulin but others do not, all assays need to be evaluated for this. However, patients with T1 diabetes do not produce significant proinsulin. Assays should have low cross-reactivity with proinsulin, how low is an arguable point. Proinsulin and C-peptide clearance in plasma are similar with half lives of ~20-30 minutes compared to ~5-7 minutes for insulin. S. Narayanan said that CAP spikes with 52-68 C-peptide since 33-63 is unstable, some antibodies will not detect this, isn't it important to consider the antibodies being used in the different assays? For example the new Tosoh assay is very specific for the two ends of the molecule and will thus not detect 52-68. R. Little said one of the reasons we have serum SRMs, to avoid matrix issues. We included the CAP sample in one of our comparisons and it actually looked pretty good but nonetheless we want to include at least one serum sample in the surveys. D. Stein said he was not aware that CAP was using something other than intact C-peptide to spike their samples, this is problematic. K. Van Uytfanghe agreed noting that this means you are measuring a fragment rather than actual C-peptide which means the results are not useful. S. Narayanan said she has found this to be the case with other manufacturers that make control materials, generally they do not use intact C-peptide. J. Eckfeldt said that these kinds of matrix issues are common with proficiency testing materials. A. Saenger said the point of PT is not always to assess accuracy but to compare within peer groups. R. Little said we would like to include serum in CAP surveys and for educational purposes we could even use this to show how matrix effects occur with the processed materials.

New labeled standard

K.. Kabytaev noted that the University of MO lab has a new isotope-labeled C-peptide standard. It is labeled with C^{13}, we will be perfoming comparisons with the NY lab (D. Stein) standard. The new standard is less expensive to synthesize and we may be able to avoid some potential interference issues.

4) Accuracy of C-Peptide Measurement Procedures: Data from the 2014 CAP Y-Survey—John Eckfeldt

- CAP has an interest in trying to develop more accuracy-based surveys using matrix appropriate materials like the CAP GH2 HbA1c survey.
- Caveats in Interpreting Y-Survey Data
  - The CAP Y-Survey uses "processed" human plasma which has been manipulated significantly and has many additives for establishing appropriate concentration of many other measurands (analytes)
  - This may lead to some degree of non-commutability of the materials with many of the clinical immunoassays
  - The proposed plan is to send out one or two off-the clot, fresh frozen human serum pools in an upcoming Y-Survey challenge, hopefully in 2016, to test accuracy of C-peptide assays in the field
- Consideration in Preparing Commutable Y-Survey Wild Card Samples
  - What are the appropriate target C-peptide concentrations for these two pools
  - What sort of donors and what situation would be used to collect serum to prepare pools with appropriate C-peptide targets (e.g., normal volunteers fasting and non-fasting)
  - Other considerations in making the pools (e.g., need for blood borne virus testing, etc.)

CAP C-peptide survey—use of serum

J. Eckfeldt noted that stability of the analyte is an important consideration in PT surveys, also how many labs are performing the particular test. The CAP Resource Committee will likely approve the plan to send fresh frozen serum samples in an upcoming survey, we have done so for other analytes including cystatin C. The commutability of reference materials is not well understood by many people and has caused problems in the past. It was noted that insulin is also in the same survey, R. Little said this would also be useful to look at. The University of MO lab measured insulin with the routine assay as well as C-peptide in the last comparison and the two were well-correlated so in terms of finding donors low and high levels of both could be obtained from the same individuals although this might not be the case for the other analytes included in that survey. J. Eckfeldt said CAP has discussed broadening out the concept to include more analytes, one problem is stability, CAP does not like to ship on dry ice. R. Little said we have some data on C-peptide stability and we could also look at manufacturer inserts. D. Stein said that either EDTA plasma with aprotinin or serum would be the best but either would need to be frozen. J. Eckfeldt said Solomon Park is capable of obtaining donors and processing the volumes of specimens needed for a large survey, but freeze-thaw cycles can be an issue if you want to do targeted pooling. For cystatin C we were able to obtain and process the specimens in MN. S. Narayanan asked if Solomon Park can get abnormal donors as they usually just draw normals. J. Eckfeldt said the logistical issues will need to be discussed as well as who will pay. CAP is interested in doing this, it used to be that PT was used mainly to see if manufacturer instructions were being followed based on peer group comparisons. Now there is much more interest in harmonization and improving accuracy. Harmonization to targets is clinically important because the typical clinician does not understand that there is a lot a variation in results among methods for many analytes. R. Little said it would be good to include serum early on in the process to see how things look at baseline, then also later on the process when we've resolved the calibration issues. With HbA1c CAP has to collect samples from people with diabetes and pool them. J. Eckfeldt said that when the HbA1c whole blood survey began they were concerned about blood type mismatches when pooling the samples but it turned out that it did not matter. R. Little said we have already validated the use of pooled serum to harmonize C-peptide. J. Eckfeldt asked what kind of stimulated C-peptide levels would be expected in normal subjects. D. Stein said 3-10ng/ml, J. Eckfeldt suggested that it might be possible to get a good range from fasting and stimulated normal subjects. M. Steffes said Solomon Park can give glucose soda to stimulate C-peptide. R. Little said we would likely still need to include some diabetic subjects to obtain the really high levels. J. Eckfeldt said CAP is now looking at other vendors for preparing matrix-appropriate survey materials, one reason it has taken CAP a while to pursue this is cost. If they have great materials but no one wants to purchase them it does not help the situation in the field. It needs to be cost competitive and labs need to understand the value of it, if you look at the current situation ~10% of the labs actually participate in accuracy-based grading, most labs just want to pass PT and it is easier to do so with peer-group grading. R. Little said there has to be some other motivation, with HbA1c there is still PT testing with materials that are not useful. D. Stein asked if the insulin PT survey has the same problem as C-peptide where the material is not authentic. S. Narayanan said for C-peptide Tosoh asked CAP about the peptide used to spike the samples and were told it was the 52-68, Tosoh noticed because their assay wasn't able to get results. We have had discussions as to whether they would be willing to use real (33-63) C-peptide, they said it depends on the number of participants. Right now the Tosoh group will all read below detection so they will all pass based on their peer group. They also proposed a separate sample exchange program where Tosoh could

send native serum to their customers. CAP is willing to do the statistical analyses and provide the information to users, but they can only do this for a limited group of customers. R. Little asked how many Tosoh customers currently participate in the C-peptide survey, S. Narayanan said ~10. R. Little said the University of MO lab currently does a periodic serum comparison with another lab using the Tosoh method, perhaps we could expand this to the other labs until we have a new survey that incorporates serum. K. Van Uytfanghe re-iterated that it is not scientifically valid to evaluate C-peptide measurements with a material that is not C-peptide. S. Narayanan said this is a problem with the Bio Rad material as well, but they are willing to switch to an intact synthetic C-peptide, at least for the EQAS survey. D. Stein asked about Randox, S. Narayanan said their material has the same problem. S. Marivoet said they are trying to change as well. R. Little said it is important for users to understand this issue. S. Narayanan said this is a problem with the Lyphochek QC materials; they cannot be used with the new Tosoh assay for that reason. They do have a different Lyphochek that is spiked with the real material but it is more expensive.

5) Insulin Standardization Update—Michael Steffes, Valerie Arends and Danni Li

- Proinsulin synthesized and then converted in the beta cell to insulin and connecting peptide (c-peptide). Insulin and c-peptide secreted in equimolar amounts.
- NIBSC now has a new insulin reference material (WHO) that is available for insulin manufacturers and laboratories.
- Why Measure Insulin?
  - Circulating concentration may be interpreted as an index of insulin secretion and action in suspected type 2 diabetes mellitus
  - Alignment among methods and thus laboratories will increase the appeal of utilizing the assay to inform diagnosis
  - Aligned insulin concentrations will improve assessment of treatments
- Modern Methods: MS
  - Currently less amenable to automation
  - Require more technical expertise, but less than previously
  - May distinguish the different insulin molecules – thus possibly enabling therapeutic monitoring
- Publications
  - Comparison of 11 Insulin Assays: Implications for Clinical Investigation and Research (Manley et. al, Clin Chem 53:5 922-932, 2007)
  - Standardization of Insulin Immunoassays: Report of the American Diabetes Association Workgroup (Marcovina et. al, Clin Chem 53:4 711-716, 2007)
  - Toward Standardization of Immunoassays (Miller et. al, Clin Chem 55:5 1011-1018, 2009)
  - Isotope Dilution Mass Spectrometry (IDMS): Innovative application to measurement of insulin in serum/plasma (Uytfanghe et al, Rapid Comm Mass Spec 21: 819-21, 2007)
  - Quantitative Insulin Analysis Using Liquid Chromatography-Tandem Mass Spectrometry in a High-Throughput Clinical Laboratory (Chen et. al, Clin Chem 59:9 1349-1356 2013) (Quest Nichols Institute)
- Insulin Standardization: Current
  - We have shown the feasibility of calibrating routine assays to a IDMS reference method developed by L. Thienpont's group using individual donor sera.
  - There are several labs including ours in MN that are developing MS assays for insulin.
  - Proposed resources
    1) Aliquots from single donors and pools with values-assigned from the 2009 study (Thienpont laboratory) and other laboratories.
    2) Additional aliquots with values-assigned by two or more laboratories
  - Suggested Process for Standardization
    1) Obtain pure human insulin to use as a calibrator
    2) Once assay is established utilize our aliquots to confirm standardization

Discussion:

M. Steffes noted that V. Arends and D. Li are developing a MS assay for insulin at the University of MN lab, and that alignment of the assays should lead to wider use of the test. Another aspect of MS assays is that we can measure not only endogenous insulin but the various forms of injected insulin as well. D. Lewis asked about the age of the NIBSC standard, M. Steffes said he believes it is recombinant material but is not sure of the age but can

check. J. Eckfeldt asked if any of the insulin reference methods/labs, including the Ghent lab, are listed with JCTLM, M. Steffes said not yet. J. Eckfeldt and R. Little noted that a comparison between at least two labs is needed to obtain a JCTLM listing. M. Steffes said they already have the data from the Ghent lab and can use this retroactively for comparison to a new lab. The MN laboratory is developing an assay as is D. Stein's lab. R. Little said that for C-peptide we were able to publish a comparison between the University of MO and NY labs and use this to get listed in the JCTLM database, but it took over a year to get the listing approved. D. Stein asked what NIBSC is charging for the material; M. Steffes was not sure. Since it's mainly geared toward insulin manufacturers it is sold in large quantities, which creates challenges in terms of cost, weighing it out in anhydrous conditions, etc.

R. Little noted that a laboratory in Japan now has a reference method and material for C-peptide, we have obtained some of this material and are in the process of performing comparisons to this lab. The issue now is to obtain a final assigned value for a reference material to use for the reference method; we should have this soon. As with HbA1c, once the final numbers are determined and are used to re-calibrate the assays we want to avoid changing them again.

RL thanked everyone for their attendance, the meeting was adjourned at 10:00 AM.

Minutes prepared by Curt Rohlfing, 9/02/15, modified by R. Little 9/2/15.

C-peptide minutes addendum:

There was a lot of confusion at the end of our July meeting regarding a new primary reference material for c-peptide. There are actually 3 different materials that have been or are being evaluated. After a bit of research, here is what I have determined based in part on communications with Robert Wielgosz and Ralf Josephs (BPIM) and Chris Burns (NIBSC).

1. The material that our group (DDL and Stein) were referring to, that was sent to Dan Stein's lab for analysis, was from BIPM (International Bureau of Weights and Measures). As it turns out, the purpose of this material is for assessing the measurement capabilities of NMIs (National Metrology Institutes). It is not a certified reference material and cannot be used as the basis of a traceability scheme (BIPM rules). The results of this value assignment and comparison will not even be available until mid to late 2016. Furthermore, the NMI in China will eventually be issuing a subset of the material that could later be used as a Certified Reference Material but this won't happen until end of 2016. This material will eventually be listed with the JCTLM.

2. At about the same time that the BIPM material was being sent out, the NIBSC also sent out materials proposed to be the next WHO International Standard. Phase III of these studies was an assessment of the commutability of this material in current immunoassay systems. This is the material that was discussed by Hanna Ritzen from Mercodia. This material will not be listed with the JCTLM.

3. The NMI in Japan (NMIJ) has already issued a pure c-peptide certificate reference material. We have done some comparisons with this material and with the Japanese Laboratory/Reference Method. This will be the best choice for our higher level standard (primary reference material) since it is in process for listing with the JCTLM and it is available for use.