2012 C-Peptide Standardization Manufacturer Meeting
Minutes

Wednesday July 18 8:00 AM – 10:00 AM
Westin Bonaventure, Los Angeles, CA

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

Manufacturer Representatives
Margherita Banci—IDS Ltd
Sean Conley—Alpco Diagnostics
Jacques de Jong—IDS Ltd
Robert Gunnarsson—Mercodia
Sachiyuki Hasegawa—Tosoh Bioscience
Russell Jarres—Alpco
Hanna Johansson—Mercodia
Yasunobu Masuda—Kyowa Medex (Siemens)
Shanti Narayanan—Tosoh Bioscience
Chisato Okamura—Fujirebio

Guests
Kelly Chun—LabCorp
Shawn Connolly—University of Missouri
Lennart Friis-Hansen—Steno Diabetes Center
Deanna Gabrielson—University of Minnesota
Hwashim Lee-- Korea Res Inst of Standards and Science
Bei Xu—National Institute of Metrology-China
Steve Phagou--LabCorp
K. Ramakrishnan—ProdConcepts/AACC Ind. Div.
Curt Rohlfing—University of Missouri
Michael Steffes—University of Minnesota
Alexander Stoyanov—University of Missouri

1) **Welcome and Introduction—Randie Little**
R. Little welcomed those in attendance, those present introduced themselves. The minutes of the 2011 meeting were approved.

2) **Update on Clinical Trials for Diabetes Prevention—Daniel Stein**
- Main points
  - > 1.4 million in the US; incidence rates rising
  - Type 1 diabetes is (usually) an autoimmune disease.
  - Adolescent (obese) Type 2 Diabetes rapidly increasing.
  - It 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.
- Natural History of Type 1 Diabetes
  - Genetic predisposition
  - Insulitus/beta cell injury in response to a putative environmental trigger
  - Cellular (T cell) autoimmunity
  - Humoral autoantibodies (ICA, IAA, Anti-GAD65, IA2Ab, etc.)
  - After 80-90% loss in beta cell mass, enter a pre-diabetes phase followed by clinical onset of diabetes (fasting and post-prandial hyperglycemia).
- Insulin secretion in-vivo
- 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 mass?

- Prevent short-term complications (hypoglycemia)
- Prevent long term complications (retinopathy, nephropathy, neuropathy, etc.)
- Diabetes Control and Complications Trial: Type 1 subjects that had some preserved beta cell function had less diabetes complications.

- 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.
- Autoimmune therapies: Bad News: Two Phase 3 Anti-CD3 Trials in New-Onset T1D Failed to Meet Endpoints
  - Teplizumab (Protégé study) MacroGenics – Humanized anti-CD3 monoclonal antibody mutated to reduce Fc receptor and complement binding. Lancet 2011, 378:487
  - Otelixizumab (DEFEND-1) GalxoSmithKline and Tolerex – Similar humanized anti – CD3 monclonal antibody. Press release.
  - Neither reached primary endpoint.
  - But, doses were low and post-hoc analysis of Protégé showed that young patients who started treatment within 6 weeks of diagnosis responded the best.
- Bio-artificial pancreas made from human or membrane-enclosed (to allow oxygen/nutrients in while preventing autoimmune response) pig/cow islets.
  - Problem: beta cells need fatty acids which do not easily move thru membranes.
  - Promising area of research
- 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)
  - Studies now standardize to 120 minute area under the curve from 4 hour MMTT.
  - Urine c-peptide (UCP)
    1) C-peptide is cleared by kidneys and ~5-10% is excreted in urine
    2) UCP previously established to track with 24h insulin secretion (Diabetol 1989 32:305)
    3) UCP/creatinine ratio (CR) accounts for dilution of urine
    4) Hattersley and colleagues have shown that the UCPCR is very stable and compares well with the MMTT
    5) Manufacturers are starting to provide reference ranges for UCPCR
    6) Hattersley and colleagues have also shown that UCPCR is very effective in distinguishing T1 from T2 diabetes.
  - Besser et al have recently shown that serum C-peptide can be used to distinguishT1 from T2 diabetes even in the presence of exogenous insulin.
- Variable Rates of Beta Cell Killing
  - In pre-T1D beta cell destruction can take years.
  - Transplant of pancreas between discordant identical twins – 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.
- Data from Joslin Clinic (Medalist study): Patients that had T1 diabetes for over 50 years and were deceased
  - 19 of 28 patients had some insulin-producing beta cells within islets
  - All of the patients had scattered insulin-producing cells
- Keenan et. al Diabetes 2010: Rough correlation between C-peptide and insulin-positive cells
- Faustman and colleagues Diabetes Care 2012;36:599-604.
  - Looked at C-peptide levels in T1 subjects using an ultrasensitive assay
  - Results showed that for a large proportion of subjects C-peptide was not detectable using the standard assay
  - 2/3 of subjects for which C-peptide was not detectable using the standard assay had C-peptide that was measurable using the ultrasensitive assay
  - There was a lot of variability among the results, this may be due to biological variability.

- The longer the duration of diabetes the less detectable C-peptide was present with the ultrasensitive assay
- However, two patients with T1 diabetes >40 years still had detectable C-peptide.

- Conclusions
  - C-peptide production persists for decades after disease onset and remains functionally responsive
  - Patients with advanced disease may benefit from interventions to preserve beta cell function or to prevent complications
- Unresolved questions
  - What is the reproducibility of ultrasensitive C-peptide assays in the same Type 1 Diabetic 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 (less 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.
- Acknowledgements
  - Peter Gottlieb
  - Carla Greenbaum
  - Barbara Davis Diabetes Center
  - Mark Pescovitz (ADA Web)
  - Type 1 Diabetes Trial Net (Jay Skyler)
  - Immune Tolerance Network (Gordon Weir)

Discussion:

Ultrasensitive Assay

MS noted that for the paper by Faustman and colleagues the standard assay (Roche) was performed at Mayo Clinic while the ultrasensitive assay was performed by the manufacturer (Mercodia) in Sweden. The University of Minnesota laboratory has looked at the ultrasensitive assay and has not found it to have a lower limit of detection than the Roche or Tosoh assays. H. Johansson responded that the investigators asked Mercodia to analyze the samples because they felt the assay performed better in their hands. After discussion with the investigators the protocol was altered to make the assay even more sensitive. M. Steffes said it was not clear in the paper that an altered protocol was used and asked if this protocol was now available. H. Johansson said it is not, they are working on further optimization and validation. D. Stein said that other investigators have had the same experience as M. Steffes and it would be good if the altered protocol could be made available as there is much interest in detecting very low levels of C-peptide. H Johansson said they just want to make sure the assay performs optimally before putting it in the hands of customers.

3) Update on Reference Laboratory Comparison Studies—Randie Little

- In 2002, the NIDDK organized a C-peptide standardization committee and funded an international comparison study of C-peptide assays.
- Phase I-IV Studies
  - Normalization with sample calibrators was effective in reducing variability of C-peptide results.
  - Normalization using patient samples with values assigned by an LC-MS reference method greatly reduces the variability among methods and laboratories.
  - Calibrators can be prepared from either single donors or samples pooled from more than one donor
  - Serum is acceptable for the Reference Method and can be used for calibrators
  - To evaluate the use of pooled serum calibrators with LC-MS assigned values for method re-calibration by the manufacturer.

- Most recent study
  - Calibrator range was 0-~3.2 nmol/L (based on the LC-MS reference method)
  - The fasting and 2-hour samples from one individual in the most recent study had unusual variability in results among methods, it turned out there were mouse antibodies present.
  - Use of the pooled serum calibrators greatly reduced variability in results among methods.
- C-peptide Reference Method/Laboratory Comparison 2011
  - 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.
  - Initial comparison data between the two laboratories in 2011 showed good correlation between them with both pooled and individual donor sera (r2=0.97 and 0.93, respectively).
- Next steps
  - Complete method setup in second reference laboratory (MO)
  - Publish comparison
  - Submit Reference method to JCTLM
  - Prepare another set of pooled sera for calibrators (#, levels?)
  - Collect another set of individual test sera
  - Ship to manufacturers for assay re-calibration
- Plan for collection of more samples: IRB approved
  - Seven levels of pooled sera, range 0.05 to 10.0 nmol/L
  - 40 single-donor serum specimens
  - 0.5mL aliquots of each
- C-peptide Reference Method / Laboratory Comparison: 2012
  - Good correlation (r2=0.9647)
  - Will continue to run comparisons

Discussion:

Measuring low C-peptide levels

R. Little said that it is not difficult to obtain serum from donor with zero C-peptide (long term T1 diabetes) but it is very difficult to obtain very low C-peptide values. D. Stein said for some of their experimental protocols they give normal individuals somatostatin to suppress beta cell function, this may be a way to obtain serum with low C-peptide. R. Little agreed that this is worth considering. R. Little said that it does not seem to matter where the comparison between the two laboratories is published to obtain the JCTLM listing. K. Ramakrishnan asked about sensitivity on the low end of the range and said that inter-assay determinations and lot-to-lot comparisons should be done to ensure the reliability of the sensitivity. If you need the cutoff to be 0.07 you probably need an interassay precision of less than 10% (results between methods/labs should differ by less than 10%). RL said at this stage our main concern is getting the methods standardized, later we can look at what results are acceptable within different ranges. K. Ramakrishnan agreed but noted that if assays really need to be sensitive in the very low range it may need to be standardized in this range now, R. Little agreed.

Calibrators

H. Johansson asked about the reference calibrators, what differences have been seen between pooled and single donor serum vs. pure reference materials? R. Little said we are talking about two different levels of materials and traceability; pure standard is used to calibrate the reference method, the secondary reference materials are patient samples. In the HbA1c world we found that use of patient samples was the only way to harmonize the different methods by avoiding matrix effects. H. Johansson agreed that a patient sample set needs to be used for harmonization but could calibrators be made using the pure materials? D. Stein said you would need to define what would be appropriate for a plasma matrix, albumin is not a plasma matrix, there are many different substances in plasma. R. Little noted that with insulin they had a pure material and found that it did not work well across all of the different assays, we have seen similar issues with HbA1c.

Reference Method and Materials, Value Assignments

L. Friis-Hansen asked how long it will take before values can be assigned to samples so that manufacturers can standardize their assays. R. Little said that samples and calibrators with assigned values have already been provided to manufacturers for the last several comparison studies. H. Johansson suggested doing a difference plot to show the differences between the two reference laboratories, D. Stein and R. Little agreed that this needs to be done, more analyses will be performed prior to publishing comparative data. D. Stein noted there is now a metrologically traceable standard available from Japan that his lab will obtain in order to further verify values for their own standards, they have already shown that their values are within 5% of the WHO standard. K. Chun asked how the target values are assigned to the comparison samples. R. Little said that in the original studies we used a routine method but since that time Dr. Stein's laboratory has assigned the values using the reference IDMS method. D. Stein said they run 3 replicates of each sample and the mean is the assigned value. We have shown that when the manufacturers methods are standardized to the reference method the between method CVs are greatly reduced; Dr. Thienpont's group showed similar results for insulin. R. Little said that initially everyone standardized to the WHO standard which was an old material that showed matrix effects, when you harmonize using matrix-appropriate serum, comparability is improved. K. Chun said that many labs dilute the WHO standard first with water, then with buffer, this may be part of the problem. The approach using native serum is good.

Reporting Units

K. Ramakrishnan asked whether the problem with different units for HbA1c measurement will be avoided with C-peptide. R. Little said we hope this is the case. An important difference is that we were using a reference method for HbA1c that was not a "true reference method" because such a method was not available at the time. At the JCTLM meeting I brought up the issue of changing reference methods, as they improve do you change the units reported in clinical care every time there is a shift in units due to a newer reference method (or material)? I do not believe the numbers for clinical care should be changed every time there is a change in the higher-order method. With C-peptide we should make sure that once we have established numbers used out in the field they should not change. Any future differences in reference method units should be taken care of higher up the traceability chain. K. Ramakrishnan asked if molarity or mass will be used. D. Stein said molarity is preferred but he still uses ng/mL due to habit, it is easy to convert between them. R. Little said this caused some confusion when working out what ranges were needed for calibrators and samples, some people were thinking in nmol/L and some in ng/mL. K. Ramakrishnan suggested consideration of reporting pmol/L as the larger numbers are easier for physicians to remember and they fit well with the scheme of reporting numbers at the low end of the range.

4) Insulin and C-peptide IDA LC/MS assay Progress Report—Dan Stein

- Ultrahigh sensitivity IDA LC/MS candidate reference method for C-peptide established (Rogatsky et al 2006)
- Harmonization of well validated C-peptide immunoassays successfully completed based upon single and pooled donor specimens
- Second site for IDA LC/MS C-peptide nearing final validation (Missouri)
- Initial IDA LC/MS insulin assay method reported (Rodriguez-Cabaleiro Clin Chem 53:1462-69).
  - Limit of Quantitation 12 pmol/L (72 ng/L)
  - CV 3.2-6.3% intra-assay, 3.8-10.3% interassay
  - Achieved goal <32% error as per ADA guidelines
- A metrologically traceable C-peptide reference material has been developed in Japan (Kinumi et al. Anal Bioanal Chem (2012) 404:13-21
  - chemical (FMOC) synthesis, HPLC purification
  - Metrologically traceable via amino acid analysis via IDMS
  - Purity by HPLC and MALDI-TOF MS
  - Minimum stability 6 months at -80C, lyophilized
  - Certified value of 80.7 ± 5.0 µg/ml; total 81.7 ± 5.1 µg/ml
  - Acceptable agreement with NIBSC 84/510 (86.6 ± 5.4)
  - Found it to be 98.7% pure, two other peaks were de-amidated C-peptide and pyroglutamate C-peptide.
  - Showed excellent stability.

- The New York and Missouri labs show excellent agreement in the latest comparison.
- We now have current generation equipment including two LC/MS/MS systems.
- Insulin Assay
  - We have obtained synthesized U-^{13}C h-insulin (mw 6064)
  - **Problem:** Insulin is susceptible to protein microheterogeneity due to deamidation
  - **Solution:** Generate stable tryptic peptide (GFFYTPK) after HPLC purification
  - Also now have an labeled internal standard (GFF$_{10}$YTPK)
  - Very good linearity but the concentration is currently pretty high.
- Future Insulin IDA LCMS Goals:
  - Purification and quantification of U-13C and peptide GFF 10KTPK h-insulin IDA standard, but need reference unlabeled insulin standard for comparison!
  - Design and optimization of IDA isotope std and plasma reformatting on automated liquid handler system
  - Optimization of on line affinity capture and trypsinization
  - Optimization of ultrahigh sensitivity 2D RP/RP LC/MS detection. Fasting insulin 3-20 µU/ml.
  - Current LOQ 14 µU/ml
    1) Goal LOQ < 2µU/ml = 12pmol/L = 72pg/ml
    2) total max CV 3% (7% at LOQ), bias limit 5%
  - Cross validation vs. Thienpont LC/MS method and other immunoassays w/ single, donor pools 10-1000pM

Discussion:
M. Steffes said that Chris Burns is working with insulin manufacturers to develop a new pure WHO insulin. Not all manufacturers can use pure materials as calibrators for their assays, so we will still need to assign values to single-donor serum or pools. D. Stein said that clinicians think in terms of µU/mL or mU/L even though chemically we are talking about pmol/L or ng/mL, this is a disconnect. The assay in terms of units is actually based on a mouse convulsion assay, it is that crude.

5) Insulin Standardization Update—Michael Steffes

- Background
  - Papers
    1) Manley et al. Comparison of 11 Insulin Assays: Implications for Clinical Investigations and Research. Clin Chem 2007;53:922-932.
    2) Marcovina et al, Standardization of Insulin Immunoassays: Report of the American Diabetes Association Workgroup. Clin Chem 2007;53:711-716.
    3) Miller et al. Toward Standardization of Insulin Immunoassays. Clin Chem 2009;55:1011-1018.
    4) Results for 10 immunoassays were not standardized despite claimed traceability to WHO 1st IRP 66/304
    5) Use of a pure recombinant insulin reference material in immunoassays was not uniformly effective to achieve harmonization of results. Some manufacturers could utilize the pure reference material. Some could not.
    6) Cross reactivity with proinsulin and split-products was minimal for most, but not all, assays
  - We have shown that providing manufacturers with serum samples with reference method values assigned by Dr. Theinpont's laboratory greatly improved the comparability of insulin results among assay methods.
- Resources from NIDDK-funded studies
  - Aliquots with values-assigned from 2009 study, Thienpont laboratory
  - Aliquots with values-assigned from Stein laboratory; currently in process
- Process for NIDDK-funded studies
  - Aliquots sent to NIH-designated laboratory
  - Results compared to assigned values by IDMS
  - This program is currently established in Washington and Minnesota

- Future of Insulin Standardization
  1) Sustain comparisons with values assigned by a reference method procedure
  2) From Minnesota supply samples to participating entities
  3) Verify results vs. the assigned values

Discussion:

Funding for Insulin Standardization

M. Steffes said only two manufacturers have provided funding; a third promised funding but has not provided it. If enough manufacturers provide funding we can provide samples and verification for manufacturers as well as laboratories performing NIDDK-funded studies, short of this it will be restricted to the latter. S. Conley said that manufacturers were asked to provide funding at the previous meeting; what is meant by there are currently two manufacturers "participating". M. Steffes responded that this means they have actually provided money. Most of the resources were originally provided by the ADA and are currently provided by NIH. If manufacturers provide more resources we can have a manufacturer-oriented program, without this we will have an assay-oriented program since NIH has provided most of the money. Manufacturers will be solicited through the IFCC. S. Conley said that his company responded to the e-mail sent out last year asking about intentions regarding funding. They were interested in participating but they never heard what the next step would be. M. Steffes said they have a program but they did not get enough offers of support to allow the program to proceed to the next stage. L. Friis-Hansen suggested that laboratories could play a role in pushing manufacturers to support the program. R. Gunnarsson asked whether AACC could provide funding. M. Steffes said they do not provide money for this purpose. R. Little noted that the insulin standardization committee is an IFCC working group, M. Steffes said IFCC does not provide money either but will be trying to solicit money. K. Chun asked if there could be a insulin certification program that could charge manufacturers for certification. M. Steffes responded that the group would like to do this but without enough resources they cannot get this started. We have many aliquots of samples and will be getting samples, and Dr. Stein's lab will be able to assign values to them, but we need more financial support to make it happen. K. Chun said that manufacturers want to participate but they want something out of it, some kind of certification that their assay is standardized. H. Johansson agreed, adding that manufacturers need to know exactly what they would get out of the program if they put money into it. M. Steffes said Dr. Greg Miller has written this up. R. Little said that we have been able to keep the C-peptide program going because we have been able to get a NIDDK grant that is combined with the HbA1c program where many of the same people are involved in both efforts.

Importance of Insulin Standardization

M. Steffes said that he is not sure what the market is for insulin. There are efforts to bring the HOMA calculation up to modern standards, it was originally devised ~20 years ago using RIA. D. Stein said that clinically 25% of the population has metabolic syndrome but physicians do not routinely measure insulin because it is not standardized. They know about insulin resistance, if they had a convenient way to diagnose it they would use it but they do not because results do not match among assays. M. Steffes said the WHO is providing a reference material for manufacturers that will be widely available. K. Ramakrishnan suggested that the best way to alarm everyone and emphasize the need for standardization of insulin might be to show how bad the CAP survey results are. D. Stein said that for insulin and C-peptide the original data showed CVs of up to several hundred percent between what were otherwise well-validated assays. This is why physicians do not order insulin tests and to a lesser degree this is true with C-peptide. Insulin is an important marker of insulin resistance; if it is double or half of a given level it makes a huge difference clinically. K. Ramakrishnan asked if C-peptide can be used as an indicator of insulin resistance, D. Stein replied that it cannot. C-peptide indicates beta cell compensation for insulin resistance, insulin feeds back based on the glucose level. As glucose goes higher it feeds back to the pancreas so more insulin is made. So it is really in the periphery past the liver, especially in the muscle, where most of the body can respond to insulin. M. Steffes added that while C-peptide indicates beta cell response, insulin is closer to insulin action.

Sample-specific Differences

H. Johansson said the 2009 paper showed that there are sample-specific differences; calibration differences can easily be corrected by standardization but how can the standardization program address sample-specific differences? M. Steffes said that it may not be able to but there are not many samples like this, how often do you see these differences? H. Johansson said they have not looked at insulin but with other ELISAs they see individual sample differences that are likely due to the antibodies, how the test is optimized, etc. These differences are not easy to address, in some cases it may involve going back to the drawing board. M. Steffes said that this tends to be a problem with immunoassays in general. D. Stein said that he sees two areas of potential error, one is that there is a small amount of proinsulin and proinsulin products, most assays are now pretty specific and in any case this probably accounts for only a few percent error. The bigger issue tends to be things like lipemia, hemolysis, etc. which can interfere with antibody affinity, this has to do with the assay itself and not the assigned value. H. Johansson agreed adding that there are other potential interferences such as heterophilic antibodies. R. Little said the emphasis right now is on bringing the methods closer together overall, sample-specific interferences have to be looked at separately in terms of individual assay methods.

Sample Collection

K. Chun asked if there are plans to spike individual samples to obtain high levels, people that have very high insulin they may have underlying disease where there are high proinsulin levels, drugs, etc. that may have differential cross-reactivity. M. Steffes said they tried to address that issue by recruiting obese people without diabetes that were not on meds, some were given orange juice to raise their insulin. The levels were not necessarily as high as we see in patients with diabetes; we are hesitant to spike samples because it could create a matrix effect with some assays. R. Little noted that for C-peptide samples are collected at the University of Missouri after obtaining IRB approval. The subjects are given a meal tolerance test where a 2-hour sample is obtained. Subjects are screened to obtain the levels we want prior to having them come back for the actual sample collection. M. Steffes said we are dealing with two different populations; with insulin you want a high value, with C-peptide you want to optimize at the low end of the range

Standardization of other Analytes

M. Steffes said there is increasing interest in glucagon now as there are more improvements in diabetes management but it is not standardized. K. Ramakrishnan asked if there is any consideration of a program for GLP peptides. M. Steffes said he has not thought about it, D. Stein said the pharmaceutical industry is showing a lot of interest in this. They are larger peptides than C-peptide, GLP-1 is ~4000 MW and tends to undergo a two-amino acid clip when metabolized and degrades rapidly. There is a lack of good assays for GLP-1, intact as well as total. M. Steffes asked if GLP-1 can be approximated by insulin combined with glucagon. D. Stein did not think so, there is too much inherent variability. It is a separate measurement with separate physiological questions and associated diseases. There is pharmaceutical interest for research purposes but clinically, there are very few instances where it would need to be measured. L. Friis-Hansen said that their lab also maintains a very old proinsulin assay, the rabbit died years ago, they can last a few more years but there are very few commercial assays available for this analyte, most are homebrewed. D. Stein said that there are few clinical instances where you would want to measure proinsulin, there is more interest in research. The beta cell will make proinsulin but then also process it into insulin; a lack of processing is felt to be consistent with beta cell deterioration. It is a larger protein which makes it more analytically complicated. R. Little noted that a few years ago the AACC sponsored a large harmonization meeting, one of the groups that was formed is looking at prioritization of analytes for harmonization. H. Johansson said it is important that other standardization/harmonization programs take advantage of the work that has already been done with HbA1c, insulin and C-peptide. R. Little said that all of the groups that are looking at standardization are aware of the current efforts.

RL thanked everyone for their attendance, the meeting was adjourned at 10:00 AM
Minutes prepared by Curt Rohlfing, 8/7/12, modified by Randie Little 8/9/12.