

2021 C-Peptide Standardization Manufacturer Meeting

Minutes

Wednesday March 17 9:00 AM – 11:30 AM US CST
Virtual Meeting

Participants:

C-peptide Standardization Committee Members

Beena Akolkar—NIDDK
Randie Little—University of Missouri
W. Greg Miller—Virginia Commonwealth University
Salvatore Sechi—NIDDK

Committee members not present

Carla Greenbaum—Benaroya Research Institute
Gary Myers—AACC
Jerry Palmer—University of Washington
Kenneth Polonsky—Washington University
Daniel Stein--Albert Einstein College of Med

Manufacturer Representatives

Kimberly Arnold--Siemens Healthineers
Philip Bryan--Ortho Clin Diag
Carol Dauscher--Siemens Healthcare
Theodora Davy--Diasorin
Jessica Giehl--Roche Diagnostics
Carissa Jones--Merckodia
Kristina Kueper--Roche Diagnostics
Stefaan Marivoet--Tosoh Bioscience
Wataru Motosuchi--Fujirebio
Shanti Narayanan--Tosoh Bioscience
Maria-Magdalena Patru--Ortho Clin Diag
C Randy Reamer--Siemens Healthineers
Hanna Ritzén--Merckodia
Michael Wunderlich--Roche Diagnostics

Guests

Valerie Arends--Univ of Minnesota
Eric Bean--Diabetonomics
Shawn Connolly--Univ of Missouri
John Eckfeldt--Univ of Minnesota
Dan Holmes--St. Paul's Hosp, Vancouver
Andy Hoofnagle--Univ of Washington
Kuanysh Kabytaev--Univ of Missouri
Tomoya Kinumi--Natl Inst of Adv Ind Sci and Tech
Anna Lam--Univ of Alberta
Michael Lawton--C-Path
Francisco Leon--ProventionBio
Michael McPhaul--Quest Diagnostics
Paulo Pozzilli--Campus Bio-Medico University
Violeta Raneva--ReCCS
Curt Rohlfing--Univ of Missouri
Jesse Seegmiller--Univ of Minnesota
Michael Steffes--Univ of Minnesota
Katleen Van Uyfanghe--Univ of Ghent
Gwen Wark--UKNEQAS/IFCC

1) Welcome and Introduction—Randie Little

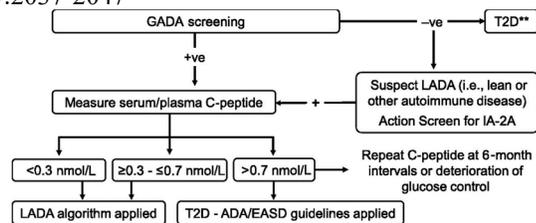
R. Little welcomed those in attendance.

2) Importance of C-peptide Standardization—Beena Akolkar

- C-peptide: Marker of Insulin Production
 - Pro-insulin is cleaved into Insulin and C-peptide (1:1); both are secreted
 - C-peptide remains in the circulation (not cleared by liver).
 - 1) C-peptide is a measure of endogenous insulin production.
 - 2) C-peptide is a better marker of insulin secretion than insulin.
- Stimulated C-peptide response can be measured in some people with long-term Type 1 diabetes
 - Preserved Proinsulin secretion in Long-Standing Type 1 Diabetes. Steenkamp DW, et al. Endocr Pract. 2017 Dec;23(12):1387-1393.
 - Beta cell function and ongoing autoimmunity in long-standing, childhood onset type 1 diabetes. Williams GM, et al. Diabetologia. 2016 Dec;59(12):2722-2726.
 - Insulin secretion measured by stimulated C-peptide in long-established Type 1 diabetes in the Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications (EDIC) cohort: a pilot study. McGee P, et al. Diabet Med. 2014 Oct;31(10):1264-8

- Assessment of preservation of beta-cell function in children with long-standing type 1 diabetes with "ultrasensitive c-peptide" method Kalinowska A et al *Pediatr Endocrinol Diabetes Metab.* 2017;23(3):130-138
- Comparison of proinsulin and C-peptide secretion in healthy versus long-standing type 1 diabetes mellitus cohorts: A pilot study Sullivan et al *PLoS One.* 2018 Nov 9;13(11):e0207065.
- Preserved C-peptide Levels Are Associated With Better Diabetes Control And Lower Risk Of Complications: Impact of C-peptide preservation on metabolic and clinical outcomes in the Diabetes Control and Complications Trial. Lachin JM, et al. *Diabetes.* 2014 Feb;63(2):739-48.
 - HbA1c decreased by 0.07% (0.8 mmol/mol; P = 0.0003)
 - Insulin dose decreased by 0.0276 units/kg/day (P < 0.0001)
 - Hypoglycemia risk decreased by 8.2% (P < 0.0001)
 - Risk of sustained retinopathy was reduced by 25% (P = 0.0010), all in unadjusted analyses
- Clinical Impact of Residual C-Peptide Secretion in Type 1 Diabetes on Glycemia and Microvascular Complications. Jeyam A, et al. *Diabetes Care.* 2021 Feb;44(2):390-398: Showed inverse relationships between residual C-peptide and
 - Daily insulin dosage
 - Mean HbA1c
 - Risk of retinopathy
 - Probability of hypoglycemic episodes
 - Hypoglycemia admissions
 - Ketoacidosis admissions
- Residual β cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. Gubitosi-Klug RA, et al *J Clin Invest.* 2021 Feb 1;131(3):e143011
 - With a diabetes duration averaging 35 years, 12.4% of the DCCT/EDIC cohort demonstrated preserved β cell function, defined with an analytically sensitive C-peptide assay as a stimulated peak concentration of over 0.003 nmol/L.
 - Concentrations of C-peptide >0.03 nmol/L remained significantly associated with lower prevalence of severe hypoglycemia throughout DCCT/EDIC.
 - Concentrations of C-peptide \leq 0.03 nmol/L were not associated with long-term benefits related to hypoglycemia, kidney disease, or diabetic retinopathy.
- Summary: Effects of C-peptide Preservation
 - The DCCT and other studies established that if even a low level of endogenous insulin production can be preserved, people with T1D suffer significantly fewer complications.
 - Higher C-peptide was shown to associate with:
 - 1) Lower risk of hypoglycemia
 - 2) Better glycemic control
 - 3) Reduction of microvascular complications
- Type 1 Diabetes Is a Predictable Disease
 - Relatives have 15x greater risk of developing T1D.
 - Stages
 - 1) Genetic risk
 - 2) Immune activation: Beta cells are attacked
 - 3) Immune response
 - 4) Stage 1: Start of T1D. Normal blood sugars, \geq 2 antibodies
 - 5) Stage 2: Abnormal blood sugars, \geq 2 antibodies
 - 6) Stage 3: Clinical diagnosis, \geq 2 antibodies
 - 7) Stage 4
 - "Prevention" and "New Onset" Trials: >2000 enrolled
- Five Therapies Preserve Insulin Secretion in New Onset (Stage 3) T1D
 - Alefacept (anti-CD2)
 - Abatacept (CTLA4-Ig)
 - Rituximab (anti-CD20)
 - Teplizumab (anti-CD3)
 - Low dose ATG (anti-thymoglobulin)
- Trialnet Clinical Trials

- Numerous trials have been completed, others are ongoing and some are planned for the future.
- Some involve ITN or DirectNet
- Teplizumab at Stage 2
 - First trial to robustly demonstrate that progression to clinical T1D can be delayed – by a median of 2 years
 - Breakthrough therapy designation (ProventionBio)
 - One of 2019's Top Advances: Endocrine Society and Nature Medicine
 - Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *N Engl J Med* 2019;381:603-613.
- C-peptide Measurements Help Distinguish Type 1 Diabetes from Monogenic Diabetes and Type 2 Diabetes
 - Impact of routine clinic measurement of serum C-peptide in people with a clinician-diagnosis of type 1 diabetes, Foteinopoulou E et al *Diabet Med.* 2020 Nov 1:e14449.
 - Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18-50 years. Lynam A, et al *BMJ Open.* 2019 Sep 26;9(9):e031586.
 - Non classic presentations of a genetic mutation typically associated with transient neonatal diabetes. Devaraja J, et al. *Endocrinol Diabetes Metab Case Rep.* 2020 Feb 26;2020:19-0125
 - The wrong diabetes: how C peptide testing might help *BMJ* 2019;365:l4352
- C-peptide Measurements for LADA Diagnosis: Management of Latent Autoimmune Diabetes in Adults: A Consensus Statement From an International Expert Panel Raffaella Buzzetti et al *Diabetes* 2020;69:2037-2047



- Fasting C-peptide, a biomarker for hypoglycaemia risk in insulin-naïve people with Type 2 diabetes initiating basal insulin glargine 100 U/ml. *Diabetes, Obesity and Metabolism*;2019, 315-323
- C-peptide Measurement in T2D
 - Dual GIP and GLP-1 Receptor Agonist Tirzepatide Improves Beta-cell Function and Insulin Sensitivity in Type 2 Diabetes. Thomas M et al. *J Clin Endocrinol Metab* 2021, 388-396
 - The utility of assessing C-peptid in patients with insulin-treated type 2 diabetes: a cross-sectional study. Dario T et al. *Acta Diabetol* 2020
 - Plasma C-peptide and Risk of Developing Type 2 Diabetes in the General Population. Sokooti S et al. *J Clin Med* 2020 Sept 17;9 (9): 3001
 - Considering Insulin Secretory Capacity as Measured by a Fasting C-peptide/Glucose Ratio in Selecting Glucose-Lowering Medications. Fritsche et al *Exp Clin Endocrinol Diabetes* 2020 Sept 18
- Importance of C-peptide Measurement
 - Measurement of C-peptide under standardized conditions provides a sensitive, well accepted, and clinically validated assessment of beta-cell function and we need to continue to focus on the lower C-peptide range (0-0.2 nmol/L).
 - Many people with Type 1 diabetes still have residual insulin secretion many years after diagnosis
 - Available data demonstrate that even relatively modest treatment effects on C-peptide will result in clinically meaningful benefits.
 - C-peptide measurement is a quantitative and well-characterized primary outcome for clinical trials of therapies aimed at preserving or improving endogenous insulin secretion in type 1 diabetes patients
 - Screening with C-peptide may be helpful in making the correct diagnosis to ensure the people get the right treatment

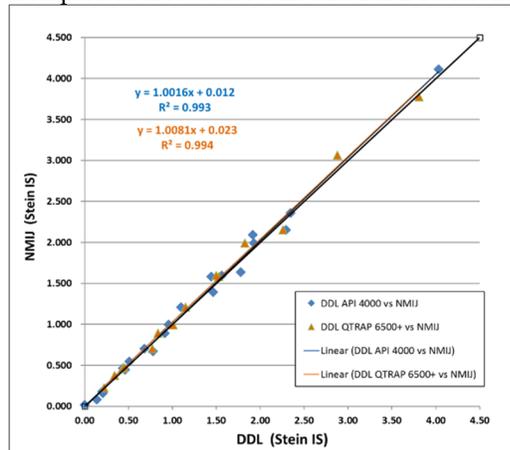
Discussion:

Clinical Use of C-peptide

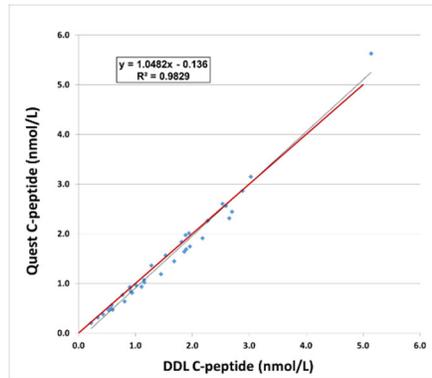
P. Pozilli asked why, despite strong evidence, C-peptide is not being used for characterization of diabetes in most clinical centers around the world. It may help considerably in determining the best therapy for hypoglycemia. R. Little asked if there is anything regarding C-peptide in the clinical guidelines. P. Pozilli responded that there is nothing mentioned, but for example, we know that for T2D patients with high C-peptide there is no need to give insulin, regardless of their glycemic control. The patient should be removed from insulin, and be educated and possibly put on other medications, but this is currently not done in clinical practice. R. Little suggested that this should be added to current guidelines, P. Pozilli agreed. C. Jones noted that Mercodia tried to get this message across at an Edelman's Taking Control of Your Diabetes event, but they hit a wall when trying to explain the use of C-peptide to physicians. She agreed that it needs to be in the guidelines, as it is otherwise difficult to bridge the gap between research and patient treatment. R. Little agreed, noting that busy physicians generally do not read a lot of the research papers but tend to look to treatment guidelines. This should be addressed with the EASD and ADA, we currently have an annual meeting at the ADA with our clinical advisory group that is focused on HbA1c, but maybe we should do something similar for C-peptide. B. Akolkar said addressing the ADA and EASD with regard to C-peptide is the way to go, she will also address the issue with her division director. G. Wark said that in the UK C-peptide is used in treatment, but specific clinical guidelines are needed. One of the main concerns is when it should be measured after diagnosis, as there are concerns about the honeymoon period. R. Little suggested putting something together that we could use when meeting with the right people on the clinical side. F. Leon noted that for his company, which produces Teplizumab, C-peptide is an important endpoint in their trials. R. Little said there are basically two tracks for C-peptide, its use for T1D in trials and clinical use in T2 diabetes in terms of proper diagnosis and treatment. The latter really needs to get into the clinical guidelines. Both tracks are important, we just need to get the word out, and also try to standardize it before it comes into widespread use. ▯

3) C-peptide Standardization Update—Randie Little

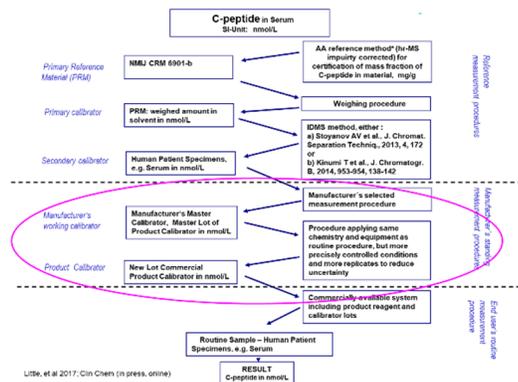
- Proposal for Standardization of C-peptide
 - C-Peptide Reference Material
 - 1) NMIJ CRM (CRM 6901-b)
 - A lyophilized synthetic peptide with high purity
 - Concentration determined by two independent amino acid analyses using liquid and gas phase hydrolyses.
 - Is listed in the JCTLM database
 - 2) Backup C-Peptide Reference Material: Sigma – Cerilliant CRM
 - LC/MS Reference Method (listed in the JCTLM database)
 - 1) UMC DDL
 - 2) NMIJ
 - 3) Comparison Between NMIJ and DDL Reference Methods (2018)



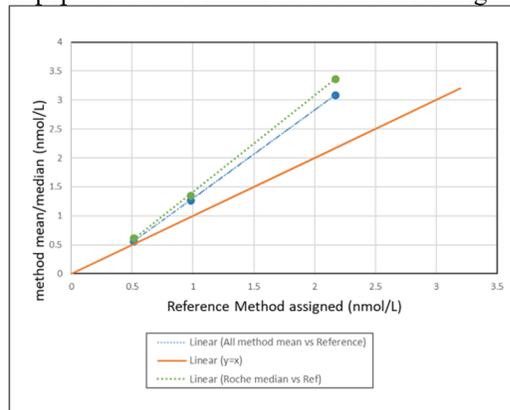
4) Quest LC/MS vs DDL (7/31/19)



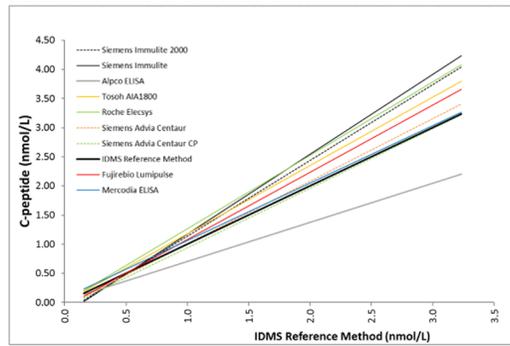
- Commutable Secondary Reference Materials Available
 - 1) 7 levels of pooled sera ranging from 0 (undetectable) to 3.77 nmol/L c-peptide.
 - 2) 40 single donor samples ranging from 0.22 to 5.14 nmol/L c-peptide
 - 3) All samples have Reference Method assigned values.
 - 4) A new set of samples is being collected
- Traceability Chain



- Publications
 - 1) Little RR, et al. Implementing a Reference Measurement System for C-peptide: Successes and Lessons Learned. Clin Chem 63:9 (2017), 1447-1456.
 - 2) Myers GL, Miller WG. Challenge to Coordinate Harmonization Activities on an International Level. Clin Chem 63:9 (2017), 1429-1430. (Editorial)
 - 3) Little RR, et al. Implementing a Reference Measurement System for C-peptide: An Addendum. Clin Chem 63:12 (2017), 1904-1905. (Letter to the Editor)
- Accuracy-Based CAP Survey: ABGIC 2020B
 - 1) 20 labs participated with 6 (+ "other") methods
 - 2) 14 labs and 3 methods appear on the report.
 - 3) C-peptide Mean/Median vs. Reference Assigned Value



4) Manufacturer's Current Calibration vs. Reference Method



- C-peptide Standardization Website: www.cpeptide.org.
- Next Steps
 - DDL: A new set of Secondary Reference Materials will be collected and value-assigned.
 - DDL: The ABGIC (or other) survey will continue to be monitored.
 - Manufacturers: It is time to recalibrate!

Discussion:

R. Little noted that the participation rate in the ABGIC survey was very low, limiting the usefulness of the data.

C-peptide samples

D. Holmes asked if the results presented are from spiked materials or actual patient samples. R. Little responded that they are patient serum samples. D. Holmes said that the subjects with high C-peptide results were likely stimulated, do we know if the results are being impacted by cross-reactivity with proinsulin? R. Little said proinsulin was analyzed on some previous samples, we didn't see issues with those. The current samples were fasting and post-meal.

CAP survey

A. Hoofnagle noted that the low ABGIC survey participation rate is because the non-accuracy based ING survey is available and fulfills regulatory requirements. We are looking at the option of including a serum sample in at least one of the ING surveys, and are also looking at other options. R. Little said that if multiple serum samples cannot be included in an ING survey, it would be good to include a low level in one and a high level in another one. J. Eckfeldt said the current thinking is to include a fresh serum wildcard sample in the ING survey, one of the questions that has come up is the cost of shipping. Data from one of the Roche methods shows stability of C-peptide at 4 degrees for at least several days, it should also be stable on MS. If manufacturers could provide data on stability at 4 degrees for their methods, it would be helpful. R. Little noted that we would need data for stability at 4 degrees after being thawed from -70 or liquid nitrogen, since that is how the samples would be processed. J. Eckfeldt said that there are several hundred labs on the ING survey, it's just that there is no impetus for them to spend the money to participate in an additional survey when the ING survey fulfills the requirements. H. Ritzen said the accuracy-based survey is important to the standardization effort, and the manufacturers have a responsibility to test stability for their assays. M.M. Patru noted that manufacturers include stability information in their package inserts; R. Little responded that they do not look at stability at 4 degrees after previously being frozen. However, if an assay does not show good stability at 4 degrees, it won't have good stability at 4 degrees after being frozen. H. Ritzen said the studies should be performed in such a way to mimic the actual conditions the survey samples will be exposed to. J. Eckfeldt noted that with the previous wildcard sample the vendor stored them for a month at -20 and the C-peptide values dropped ~20% as a result. We know that for at least some immunoassays stability at -20 is not good, probably due to ice crystals with a high concentration of salts. R. Little said that the same is true for HbA1c; -20 stability is not good.

C-peptide Assays

A. Hoofnagle said there is a research group on the west coast trying to develop a C-peptide method using protease cleavage with Glu-C that would have the sensitivity required to be useful for T1D. They are wanting to develop a SOP so that anyone could run the method, which would be a high flow LC/MS method that does not use antibodies. Comparisons among laboratories running the method and against the current C-peptide reference method are

looking very good. R. Little said that when the C-peptide standardization started, the idea was to focus on the labs performing clinical trials. However, the problem was that these labs were all using commercial methods, so we decided to try to standardize commercial methods. An alternative is what A. Hoofnagle described; developing a MS method that could be used by all of the laboratories performing clinical trials.

4) Update from Manufacturers

- Abbott Laboratories: Not present
- Alpco: Not present
- Diasorin: Theodora Davy
 - Due to the focus on Covid 19, there has been little progress on C-peptide over this past year.
 - Also, they have been busy dealing with the new IVDR regulation in Europe, which has been time-consuming
- Mercodia: Hanna Ritzen and Carissa Jones
 - Since Mercodia's results match well with those of the reference system, the only significant change for them would be the traceability chain.
 - With help from the committee, they may be able to provide information regarding standardization to their customers.
- Ortho Clinical Diagnostics: Maria-Magdalena Patru
 - Ortho has not made progress on C-peptide over the past year, due to Covid and the new EU directive.
 - There are concerns
 - 1) Regulatory issues with re-calibration
 - 2) Sample panels: Will these be updated and maintained so that we can go back to them when needed?
- Roche Diagnostics: Michael Wunderlich
 - Elecsys Manufacturing Penzberg
 - Chemiluminescence assays utilize magnetic beads, a capture antibody and a signal antibody.
 - All phases of production, including standardization quality control, are performed in Penzberg with the exception of the final packaging.
 - Standardization: Target value assignment
 - 1) Analyze a sample set
 - 2) For C-peptide, using the WHO standard
 - 3) Obtain a curve which can be used on all instruments worldwide.
 - 4) Curve is flexible and can be adjusted if necessary.
 - 5) The customer only needs to run two levels to calibrate.
 - 6) Calibrator target values are assigned using 6-8 instruments.
 - 7) Controls are provided to customers to check the calibration
- Siemens Healthineers: Carole Dauscher, Randy Reamer, Kim Arnold
 - They are currently in the process of re-standardizing to the new 13/146 standard.
 - Roche would prefer to use a white paper to describe the standardization process and give a conversion equation to users rather than including it in the IFU.
 - To include the information in the IFU would require a lot of time and resources, including regulatory and marketing assessments.
 - Like other manufacturers they are dealing with higher priority issues at the moment.
 - Until they get their IVDR paperwork back, they are in freeze in terms of assay design changes, meaning we cannot change IFUs, labeling, etc.
 - A white paper essentially divorces the standardization information from the regulatory bodies, and could be done quickly.
 - After the review period, they can consider design changes again, including additions to the IFU.
 - They would need a timeframe for when everyone would make the changes to their IFUs; if one manufacturer changes and no one else does, it would create potential confusion.
- Tosoh Bioscience: Stefaan Marivoet
 - Their Japanese colleagues have been hesitant, but they closely follow the Japanese clinical organizations.
 - The key is to get the clinical organizations to incorporate standardization recommendations in the clinical guidelines.

- Efforts to standardize TSH and T4 have been difficult due to large between-method differences, but it has already been accomplished in Japan due to clinical recommendations.
- In Europe they are compliant with the IVD Directive because they are traceable to the highest standard. If it can be shown that this standard is not fully commutable but there is an alternative that is, they can use that standard as long as traceability can be documented.
- It will be more difficult in Europe in the future as documentation for all tests will have to be reviewed by notified bodies, not just for problematic tests.
- Fujirebio: Wataru Motosuchi
 - There is nothing new to report regarding C-peptide, their situation is similar to that of other manufacturers.
 - During 2020 they have been busy with Covid-19

Discussion:

Regulatory Issues

R. Little said that FDA does not require a new submission when changing a calibration, they just need to be notified if the change is significant and there is some additional internal paperwork required. Europe is probably different. We do want to follow IFCC and other recommendations in the process of achieving standardization. J. Eckfeldt said that manufacturers need to clarify what is needed from the FDA. With cystatin C there was a significant holdup in re-calibrating assays because the FDA required a new 510K since the claims could change. With other analytes it seems to be less of an issue. R. Little said she has been in touch with the FDA. The FDA responded by saying that although C-peptide assays are regulated they are generally exempt from pre-market review. This means that if a manufacturer re-calibrates their assay, they would need to validate it but they would not need FDA clearance to put it on the market. W.G. Miller said that in his discussions with the FDA they have expressed an interest in having the manufacturers coordinate among themselves over some kind of reasonable timeframe. If this committee could coordinate a timeline with manufacturers, it would give the manufacturers a basis for incorporating the recalibration into their planning. Right now they are faced with higher priorities, but we could start laying the groundwork now with the goal of achieving this in a few years, e.g. 2023. R. Little said a few years ago manufacturers requested that the committee come up with some language to inform their customers of the change. She can send this out to the manufacturers again. The manufacturers could provide both sets of numbers over a changeover period, then start providing only the standardized results at a later date. Some manufacturers would not have to change or the change would be very small, but others will require more adjustment. S. Narayanan noted that some customers that are involved in long-term clinical trials cannot change their reported numbers in the middle of these trials, so the dual numbers would be acceptable. In terms of the FDA, C-peptide is a class 1 analyte; the manufacturers are required to have data on file and provide it if it is requested, but there is no FDA review and a new 510K is not required. The issue with dual calibrations is that there will be two sets of numbers out there, from a post-market perspective this is something manufacturers will have to manage very closely. C. Jones noted that for anything marked IVD, you can't have the same item with two different protocols selling at the same time under the same article number. If there are two different protocols, they would have to be labeled as two different products. For example, with their new glucagon assay they had to stop selling the old assay prior to launching the new one, since it was the same product. R. Little asked if it could be on paper for informational purposes. It would show the relationship with an explanation. H. Ritzen thought so, there could be information such as a technical note explaining the relationship and although the numbers would not be reported out yet, it would explain what we are moving toward. M.M. Patru agreed that the information could be reported in a white paper or something similar. However, in recalibrating the struggle will be with regulatory issues. With a new calibration the assay is considered a new product, although FDA may not require a new review, other countries will. Also, coordination between the manufacturers is a good idea, but similar efforts with phthalates and TSH have not worked well. Each manufacturer has their own priorities, and in going through the regulatory process there are decisions to change things, which can lengthen the process timewise. R. Little asked H. Ritzen and C. Jones if they could look into what would be required from their end in Europe in terms of paperwork, product information, etc. H. Ritzen said they can, from Merckodia's perspective if there is no change to the assay except for the traceability chain, this would not be much of a hurdle compared to those assay that would need to change. This is something that would need to be discussed with regulators. R. Little said that initially just providing the information and conversions might be the way to go to get everyone on the same page. For PT surveys we might need to collect both sets of results. S. Narayanan gave an example with PSA where there are two reference standards available. In the surveys these are differentiated from each other, maybe something similar could be done with C-peptide. R. Little agreed, and noted that everyone right now is standardized

to the old WHO standard which we have shown does not actually standardize due to commutability issues. H. Ritzen liked the ideas of developing a timeframe and providing letter from this group to share with customers. Right now their customers are demanding traceability to the WHO standard. A consensus is needed between manufacturers regarding how and when to move forward with standardization. From Mercodia's perspective, we currently match the reference method very well and will not need to recalibrate. However, we need to have our internal program in place to control the consistency and we will need to change our traceability chain. We also need a survey so that we can know the consistency of the traceability, and more generally do we need to make sure we have life-cycle management of the secondary reference material? R. Little said the committee can produce a letter explaining the standardization, and the published paper goes into much detail regarding both the old and new traceability chains. H. Ritzen said there are regulatory concerns and things that will need to be done internally by manufacturers, so consensus on a timeline is important. W.G. Miller said that the problem has been a non-commutable calibrator (WHO) in the traceability chain, in a sense the proposed standardization scheme basically provides a means to correct the commutability issues. If we use the right language in describing how the program is trying to fix the problem in the field, it would likely be acceptable to regulators as long as the reasoning is sound. Also we can provide something to show to customers describing why there is a problem with results in the field and explain how we now have a solution. As far as the clinical trials are concerned, as long as actual assay doesn't change it is simply a mathematical conversion where the relationship between the old and new results would be known. If we have all of these things in place, we would then have a framework for accomplishing the goal of standardization. The pieces are already there, the next step is to package it in a manner that's acceptable to the IVD manufacturers and regulators in different countries. C. Jones agreed with W.G. Miller and H. Ritzen, adding that manufacturers are working with people from academia to diagnostic laboratories, the demands and questions are different. The framework and documentation are needed in order to address the concerns of people who may need to trace their results back to the WHO standard. R. Little suggested that a small group be put together to try to address the regulatory and other issues. S. Narayanan asked if the mathematical conversion is linear across the range of C-peptide results. R. Little said that the relationships look pretty linear, we will need to look at this again with the next batch of samples because some of the assays may have shifted over time. S. Narayanan said if the relationships are linear, the manufacturers could simply include the mathematical conversion factors in their package inserts, for those performing clinical trials who need to convert their numbers. If the relationship is not linear it could be a problem. H. Ritzen added that the manufacturers need to confirm that the relationship is linear with their respective assays. We need a method in place so that all of the individual manufacturers can use it to establish the linearity of their assay and each can determine their own factor. R. Little said the manufacturers already have their data from the previous comparison and will also have new data to use for these purposes. R. Little asked M. Wunderlich if the re-calibration for Roche involved changing just two calibration points. M. Wunderlich said in principle yes, but there is much more involved in re-calibrating an assay. The biggest regulatory hurdle is not the U.S. or Europe, but China. It takes 18-24 months to accomplish the change in China, it is very complicated. It also involves changing the target values for our internal standards and changing the instructions, as well as changing product numbers. For China in particular, once a change is approved you have to immediately stop selling the previous product and begin selling the new one. It is similar in some other countries, trying to coordinate the timing of all these shipments to different countries is very difficult. K. Kueper added that the controls and calibrators would change as well, meaning that they would also need new product numbers, so they would need to apply for three new products in China. R. Little said this is why is it important to first make the conversion on paper, so that customers can at least see how the current numbers compare to the standardized results. She asked if Roche could check on the feasibility of adding the conversion information to product inserts in China when the assay otherwise stays the same. W.G. Miller said that if the assays stay the same but the end-users are provided the conversion information and could therefore decide which numbers to report, it could lead to bi-modal distributions of the reported results and confusion among healthcare providers. Depending upon how it was implemented, it could make things worse rather than better. R. Little said it would provide labs performing clinical trials to report standardized values, at this point that may be sufficient. Later on, if and when C-peptide cutoffs are incorporated into the clinical guidelines and C-peptide is more widely utilized further steps to standardize will be needed. A. Hoofnagle suggested that a paper showing how each assay performs compared to the reference method might be useful. He noted that manufacturers already release different lots of calibrators which result in shifts we've seen on the accuracy-based surveys, where results from international labs do not look the same as those in the U.S. R. Little said that we've been waiting all of these years for something to happen with standardization but it has not, so providing the information on the package inserts might be a good intermediate step. With HbA1c the ADA issued specific target values which made standardization a priority, there is currently nothing like this for C-peptide in the guidelines. If specific cutoffs eventually end up in the guidelines, there will be pressure for the clinical assays to

change. M.M. Patru asked where the information would come from, would the committee publish a paper showing how all of the assays relate to the reference, or would each manufacturer have to provide the information? If it is provided in the package insert without validation and approval it would be considered off-label use. R. Little said that the data come from the comparison studies we did with manufacturers, manufacturers have this data, so what information is needed in order to put the information in writing for customers? M.M. Patru said if it is not provided in the package insert it would need to be supplied by request. T. Davy said it would be a declaration coming out of the quality or regulatory department, without including the information in the IFU. If the information were to be put in the IFU, we would need regulatory approval and it would mean starting over. A. Hoofnagle said it sounds like the notifications they get from manufacturers regularly informing them of issues with or changes to the assay, e.g. a shift in calibration, etc. M.M. Patru said that Ortho has two LDH assays, one is standardized to the IFCC but it is simply a mathematical conversion, but they still have to have two separate product numbers. The bimodal idea is not a good solution, not just because of the different numbers but also the regulatory issues. What she would need is the data, the conversion factor and some background which the group can come up with and agree upon. The other option would be to move ahead with standardizing the assay. R. Little asked M.M. Patru if she is in favor of providing the conversion information in the IFU, M.M. Patru said she is. However, manufacturers need to check with their regulatory bodies; it likely will not be possible without doing the validation work. R. Little said manufacturers need to let us know what they need from us to accomplish this. They already have data from the previous comparison, and there will be another set of data once we have a new set of samples available. The data show the relationship between the individual method and the reference method with commutable materials. Once you have determined the conversion equation, what else is needed in order to put the information in the IFU? M.M. Patru said that if the method compares well with the reference method and adjustment is not needed, they would need to change the SOP and show the relationship with the reference method. The information could then go into the IFU, although some countries might require additional verification, she will need to check with the regulatory people at her company. Once the work is done, we would need to be able to maintain the standardization, so we would need assurance that the sample panels will be available when needed. R. Little said the sample panels will be available, the next set will be stored in liquid nitrogen while we study -70 stability further. She urged the manufacturers to discuss the matter with their regulatory people to determine what would be required to include the information in IFUs. J. Eckfeldt said that when they changed the standardization of creatinine over to IDMS, it worked pretty well, although it took a few years for manufacturers to fully implement it. With cystatin C a new international reference preparation changed the values for some assays by ~20%. Even 10-12 years later it is still confusing. The CAP surveys listed IFCC calibrated and not IFCC calibrated, and it was clear that some labs did not know what they were reporting. In discussions with colleagues it would be good to consider what was done differently, while creatinine took a relatively short time Cystatin C has been going on for years and is still not done. Some laboratories still are confused about what they are reporting, and thus the healthcare providers are putting the wrong numbers into EGFR calculations and getting the wrong answers. R. Little said hopefully this will not be an issue with C-peptide.

IFU changes

R. Little said manufacturers changing their IFUs at the same time is probably not critical, since it would just be providing information and labs would not immediately change their reported values. C. Dauscher asked why a white paper would not be sufficient if all that is being done is providing information. R. Little asked how customers respond to white papers vs. information in the IFU. C. Dauscher said it's a balance with the regulatory issues, some things they would consider to be minor changes can be interpreted by regulators as major changes. R. Little said her impression was that the regulatory issues with adding IFU information would not be a problem, with the possible exception of China. C. Dauscher said she would need to verify that, R. Little suggested it would be good for other manufacturers to do so as well. R. Little asked S. Marivoet how he felt about providing information and conversion factors to customers. He did not think it would be a major issue because for example, with prolactin they already provide two conversion factors. The standardization should not be an issue for labs performing clinical studies, as long as they are provided with the conversion equation so they can go back to the old numbers for studies already in progress. If customers start demanding the assays be standardized it will happen. However, right now their customers are not complaining, and there are many more pressing issues to address with the upcoming 2022 directive.

Japan

R. Little said she has been in touch with a clinical person in Japan on and off, they were supposed to present something but she hasn't heard from him recently. She does not know how to interface with the Japanese clinical

societies, but has tried. S. Marivoet said his Japanese colleague was unable to attend this meeting, but he will have them contact R. Little. R. Little said this was done before through Tosoh, and she sent some presentation materials to the Japanese contacts but she does not know what happened.

R. Little said that she previously had trouble contacting someone from Fujirebio, therefore they were not part of the previous sample exchanges. She asked W. Motsuchi if the DDL could send a panel of samples to them in the near future, he agreed.

Future plans

R. Little said that at this point it is important that manufacturers check with their regulatory divisions regarding the possibility of putting conversion information in white papers or IFUs. She would like to then revisit this issue. W.G. Miller said that there seems to be consensus that a coordinated plan that includes a timeline would be well-received. The exact contents of this plan will require further discussion. A. Hoofnagle asked about getting someone from FDA involved, R. Little agreed that it would be a good idea. W. G. Miller suggested getting regulators from other countries involved, especially China. R. Little said it might be difficult to get in touch with the right people. Alternatively, someone from a manufacturer regulatory division that is familiar with the situations in different countries might be a good substitute. She is open to suggestions for people that would be familiar with the regulations in Europe and/or China. S. Marivoet said that he will see about involving someone from MedTech, the organization that represents all of the manufacturers in Europe. H. Ritzen suggested that R. Little resend the previous comparison data, they need to re-examine it to see if there would be any design changes needed. If there are no changes to the assay and it's just a conversion factor, it should not be a major issue but if the assay is affected in terms of the calibrators, or if it affects the sensitivity or dynamic range, that would be a larger issue in terms of regulatory requirements. R. Little said that right now it appears the actual recalibration of the assays is more of a long-term goal, the immediate goal is to get the conversion information included. Although it does not appear that the assay recalibration would be a major regulatory issue in the U.S., it could be in some other countries. M.M. Patru asked if there could be a paper published based on the comparison data if all the manufacturers agree, because it is no problem for them to provide publications to customers. R. Little said this is something we can discuss with each company. M. Lawton was supportive of the re-calibration effort, the Critical Path group is leading a T1D consortium whose goal is to build a clinical trial simulation tool. The tool is based on integration of diverse data types, including clinical data from Prevention Bio and observational studies. C-peptide data are key to building this tool, so to the extent that C-peptide data could be comparable across different studies it would be extremely helpful to this effort. C. Path also has frequent interactions with EMA and FDA, so they can suggest some additional contacts. R. Little said that would be helpful, and it would be good to hear more from C. Path in future meetings.

It was agreed that another meeting will take place in three months. R. Little thanked everyone for their attendance, the meeting was adjourned at 11:30 AM US CST.

Minutes prepared by Curt Rohlfing 04/16/2021. Modified by R. Little 4/19/2021.