2025 C-Peptide Standardization Manufacturer Meeting
Minutes
Thursday, November 13 10:00-11:40 AM CST (Virtual Meeting)

Participants:

C-peptide Standardization Committee Members
Kuanysh Kabytaev--Chair, Univ of Missouri
Andrew Hoofnagle--Univ of Washington
David Leslie—St. Bartholomew's Hosp UK
Paolo Pozzilli--Campus Bio-Med Univ of Rome
Robert Wielgosz--BIPM, France
Beena Akolkar--Liaison, NIH/NIDDK

Committee members not present
Salvatore Sechi--Liaison, NIH/NIDDK

Guests
Shawn Connolly--Univ of Missouri
Lutz Heinemann--dia team
Ralf Josephs--BIPM
Stephen Kahn- Univ of Washington
Gustavo Martos--BIPM
Masami Murakami--Gunma Univ Japan
Curt Rohlfing--Univ of Missouri
David Sacks--NIH
Erwin Schleicher—Univ Hosp Tübingen
Gwen Wark--NHS

Manufacturer Representatives
Aya Asagoshi—Tosoh Bioscience
Ming Bangfa--Mindray
Paul Barto--Quidel Ortho
Andrea Battola—Tosoh Bioscience
Marvin Berman—Abbott Diagnostics
Jean-Sebastien Blanchet--Beckman
Maurizio Borla--Diasorin
Susan Brophy—Abbott Diagnostics
Stewart Cristan--Beckman Coulter Diag
Jamie Deeter--Roche Diagnostics
Jean-Michel Garcia—Tosoh Bioscience
Karen Gignac—Abbott Diagnostics
Claudia Gonzalez--Tosoh Bioscience
Yanlin Han--Autobio
Suguru Hayama—Miraca Life Sciences
Yuka Imai—Miraca Life Sciences
Michael Kjome--Beckman Coulter Diag
Stephan Marivoet—Tosoh Bioscience
Tiia Maxwell--Siemens Healthineers
Maria Vittoria Messina--Campus Bio-Med Univ of Rome
Godwin Ogbonna--Quidel Ortho
Aya Oshimo—Miraca Life Sciences
Miguel Sainz--Mercodia
Ana Shulla-Mesi--Abbott
Christian Vogl—Roche Diagnostics
Mie Wakabayashi—Miraca Life Sciences

1) **Welcome and Introduction— Kuanysh Kabytaev**
   Kuanysh Kabytaev welcomed those in attendance and acknowledged the leadership of previous chair Dr. Randie Little who recently retired. Participants introduced themselves and the 2024 meeting minutes were approved.
2) **Clinical Update— C-peptide: Who, When and Why?—David Leslie**
   - Spectrum of Diabetes
     - Pre-Type 1 Diabetes: Disrupted Dialogue
       1) Dialogue between beta cell and immune response
       2) Immune response against autoantigens derived from the beta cell
       3) We now know these changes are anti-dated by changes in both the beta cell and immune response prior to autoimmunity
     - Pro-insulin is produced in the endoplasmic reticulum.
     - About 20% of pro-insulin molecules are unfolded and are removed by the unfolded protein response system, the remainder is cleaved into insulin and C-peptide.
     - Most of the insulin secreted is removed on the first pass through the liver, so insulin measured in the peripheral blood is not a very accurate indicator of the amount secreted.
     - C-peptide is a better indicator of insulin secretion/ beta cell function as it is not extracted by the liver. It can be used for this purpose in patients on insulin since C-peptide assays do not detect insulin.
     - Relationship between insulin secretion and sensitivity: Typically think of patients with T2 diabetes as having high insulin secretion and T1 patients as having low insulin secretion.
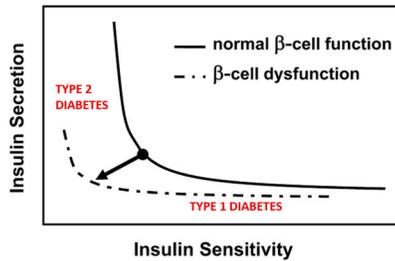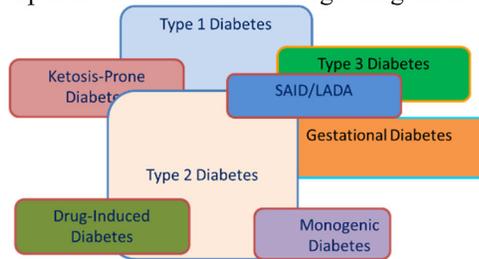
- o However, we now know there is heterogeneity within the system.
- o The Heterogeneity of Type 1 Diabetes: Implications for Pathogenesis, Prevention, and Treatment— 2024 Diabetes, Diabetes Care, and Diabetologia Expert Forum. Diabetes Care 2025;48(10):1651-1667. Doi:10.2337/dci25-0013 (also published in Diabetes and Diabetologia)
    - 1) Describes the heterogeneity of T1D and the heterogeneity of therapy
        - ▪ Immunomodulatory approaches
        - ▪ Beta cell replacement therapies including islet replacement and stem-cell based therapies
    - 2) C-peptide is the best way we have of assessing whether therapies are working.
- Spectrum of C-peptide
    - o C-peptide Falls by Age and T1D Duration (McKeigue, Colhoun et al BMC Medicine 2019, Maddoloni et al DOM 2022)
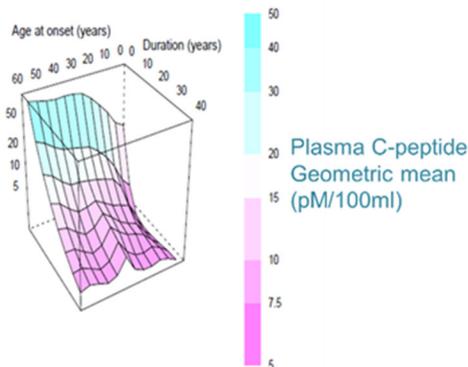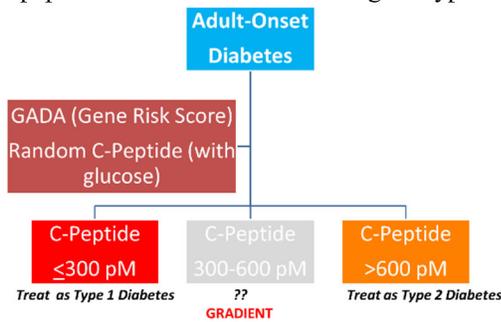

- o T1 patients that are older at diagnosis and/or have shorter duration of diabetes typically have higher C-peptide levels than patients that are younger at diagnosis and/or have longer duration.
- o T1D GRS/ Progression in Trialnet subjects (Single Ab +ve): Templeman Diabetes Care 2025
    - 1) Adults and children with family histories of T1D and are positive for a single antibody.
    - 2) Adults with the highest gene risk scores go on to develop T1D at a significantly higher rate than those with the lowest gene risk scores over 8-10 years.
    - 3) The difference between the groups is far more dramatic in children, where the high gene risk group developing T1D much more rapidly and to a much greater degree than high risk adults.
    - 4) The pattern is very different when looking at T2D gene risk scores, where for adults the risk of developing T1D is higher for the high risk vs. low risk group but for children there is no difference between the high and low risk groups.
- o Insulin secretion and sensitivity
    - 1) Young people with DR4 (T1DE1) tend to have very marked loss of insulin secretion.
    - 2) Older patients with DR3 (T1DE2) often have only a single antibody to GAD and may not even require insulin at diagnosis (autoimmune but looks like T2D).
    - 3) There are T2D patients that are severely insulin deficient (SIDD).
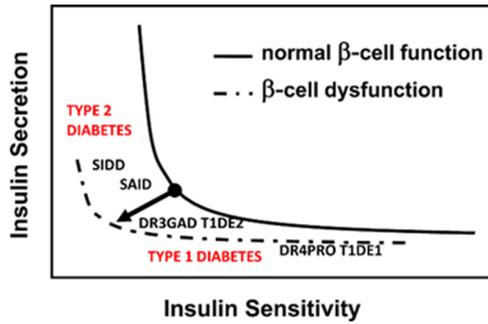
- o Categorisation
  1) The majority of T1D cases being diagnosed are adults and most do not require insulin at diagnosis.
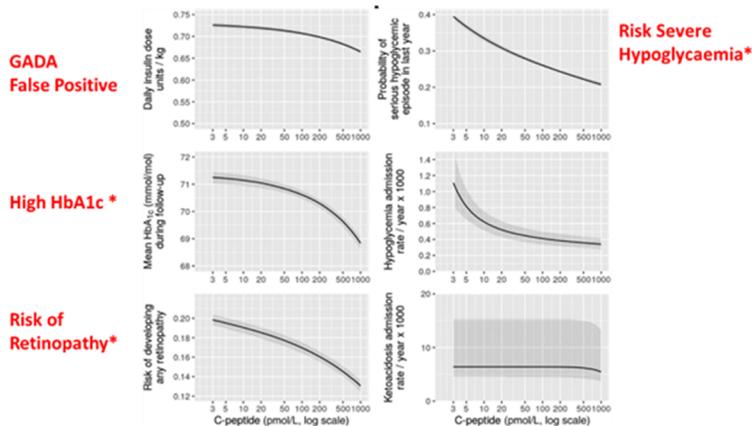 2) Options to consider when diagnosing diabetes

  3) C-peptide is valuable in determining the type of diabetes

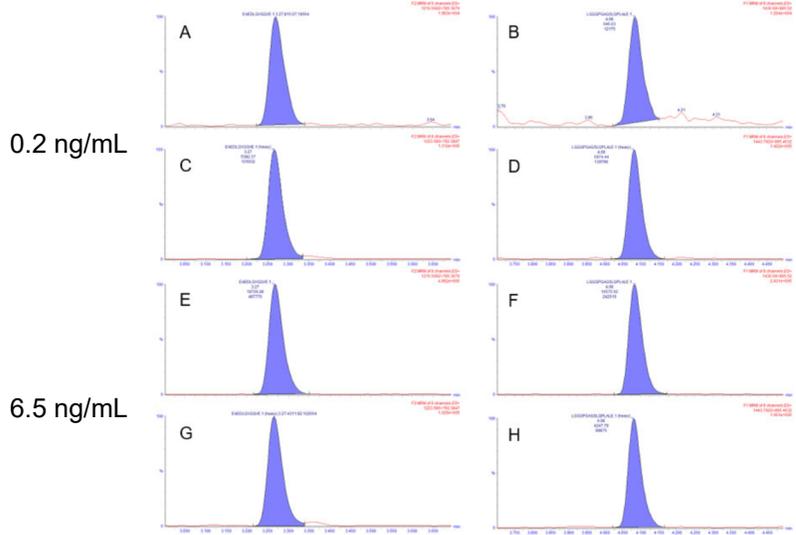
- o Prognosis: Anita Jeyam et al. Dia Care 2021;44:390-398
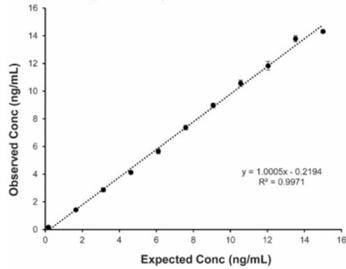
Effect of C-peptide Level at Baseline

Discussion:

P. Pozzilli said their group were the first to introduce the three categorization levels in 2020. The key point is when we measure C-peptide in a patient with diabetes, the blood glucose levels at the time of sampling may have an effect on beta cell function. It is surprising that this aspect has not been taken into serious consideration when measuring C-peptide. If it is measured when the BG levels are 160-180 mg/dL, there may be a different effect on beta cell function than if the BG levels are 100-110 mg/dL. It is important to state a precise rule for when to measure C-peptide with respect to BG levels, this issue needs to be addressed. D. Leslie agreed, but noted that the problem for the clinician is that these analytes are typically measured in a clinic setting where we cannot expect the patient to arrive fasting or precisely two hours after a standard meal. Given this situation we have presented rough guidelines as to how C-peptide should be assessed and applied in clinical practice. The three categories are simplistic, but in clinical terms that may be where we are. S. Kahn agreed with P. Pozzilli and D. Leslie, noting that the endocrine system involves feedback loops. If you have a glucose level of 250 mg/dL and a C-peptide <300pM, that C-peptide level is clearly low. But if the glucose is much lower it is less clear. We need to be careful about using these values as absolute cutoffs for everyone, which tends to be the approach. K. Kabytaev said this also illustrates the importance of standardizing C-peptide results.

2) **The TaMADOR Consortium: Interlaboratory Transfer of a Combined Antibody-free Insulin/C-peptide Assay—Andrew Hoofnagle**
   - Islet Cell Dysfunction: Hallmark of type 1 diabetes
   - C-peptide and Insulin--Investigating diabetes
     o Hyperglycemia--Residual beta cell function vs. insulin resistance
     o Hypoglycemia--Factitious or iatrogenic hypoglycemia vs. insulinoma
     o Glucose tolerance tests and clamps--Evaluate metabolic response
     o Why LC-MS/MS?
       1) Better specificity
       2) Multiplexing
     o Previous methods
       1) Measure intact C-peptide and insulin (poor ionization)
       2) Analyte enrichment
          ▪ Immunoaffinity
          ▪ 2D-LC-MS/MS
   - C-peptide and Insulin--Poor concordance (College of American Pathologists)

Insulin µIU/mL	ABGIC-04					ABGIC-05					ABGIC-06				
METHOD	N	MEAN	SD	CV%	MEDIAN	N	MEAN	SD	CV%	MEDIAN	N	MEAN	SD	CV%	MEDIAN
ALL METHODS	17	173.99	28.56	16.4	167.2	17	109.28	17.72	16.2	108.2	17	42.76	7.82	18.3	42.6
Beckman UniCel Dxl	4	-	-	-	145.2	4	-	-	-	92.2	4	-	-	-	33.3
Roche cobas e600 series/E170	4	-	-	-	200.9	4	-	-	-	126.6	4	-	-	-	51.0
Roche cobas e801/e402	3	-	-	-	203.8	3	-	-	-	125.7	3	-	-	-	50.5
Tosoh ST AIA-Pack	3	-	-	-	161.1	3	-	-	-	98.4	3	-	-	-	38.4
					33%					32%					41%

C-Peptide ng/mL	ABGIC-04					ABGIC-05					ABGIC-06				
METHOD	N	MEAN	SD	CV%	MEDIAN	N	MEAN	SD	CV%	MEDIAN	N	MEAN	SD	CV%	MEDIAN
ALL METHODS	16	14.561	2.145	14.7	15.41	16	9.592	1.333	13.9	10.14	16	4.832	0.763	15.8	5.12
Roche cobas e600 series/E170	4	-	-	-	15.63	4	-	-	-	10.38	4	-	-	-	5.27
Roche cobas e801/e402	3	-	-	-	15.90	3	-	-	-	10.70	3	-	-	-	5.41
Siemens ADVIA Centaur, Centaur XP/XPT	3	-	-	-	13.60	3	-	-	-	8.85	3	-	-	-	4.49
Tosoh ST AIA-Pack	3	-	-	-	15.78	3	-	-	-	10.32	3	-	-	-	5.06
Reference Method**		11.150					7.870					3.540			
		31%					22%					37%			

   - Proteolysis of C-peptide: A trypsin-less surface (Owusu, JMSACL, 2021)

EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ

Endoproteinase GluC

EAEDLQVGQVE
(+1 Precursor m/z 1216.57)

LGGGPGAGSLQPLALE
(+1 Precursor m/z 1436.69)

Internal Standard
EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ

- Sample Clean-up For Sensitive Detection (Owusu, JMSACL, 2021, Foulon, JMSACL, 2022)
    - Acetonitrile Precipitation
    - Dry down supernatant
    - Solid-phase extraction
    - Reduce, Alkylate, Digest
    - LC-MS/MS
- LC-MS/MS Detection After Proteolysis (Owusu, JMSACL, 2021)

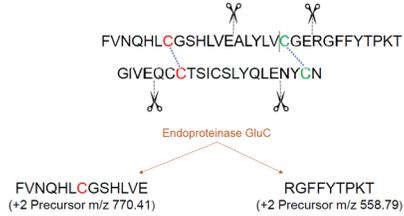0.2 ng/mL

6.5 ng/mL

- Linearity Study (Owusu, JMSACL, 2021)

$y = 1.0005x - 0.2194$
$R^2 = 0.9971$

- Precision Study (Owusu, JMSACL, 2021)

Day	Replicate[b]					Within-day CV[c]
	1	2	3	4	5	
1	0.35	0.36	0.29	0.33	0.32	8.3%
2	0.34	0.34	0.32	0.31	0.34	4.3%
3	0.30	0.34	0.35	0.35	0.34	6.2%
4	0.31	0.33	0.30	0.29	0.34	6.6%
5	0.34	0.34	0.34	0.33	0.34	1.3%
Between-day CV[d]	6.6%	3.2%	8.0%	7.1%	2.7%	Total Imprecision (%CV)[d] 7.7%

In clinical production, 10% CV at 0.16 ng/mL (N=40 batches)
- Proteolysis of Insulin--Specificity with GluC (Foulon, JMSACL, 2022)

FVNQHL**C**GSHLVEALYLV**C**GERGFFYTPKT
GIVEQC**C**TSICSLYQLENY**C**N

Endoproteinase GluC

FVNQHL**C**GSHLVE
(+2 Precursor m/z 770.41)

RGFFYTPKT
(+2 Precursor m/z 558.79)

Internal standard: fully ¹⁵N labeled insulin

- GluC works for Insulin Too--Rapid peptide release (Foulon, JMSACL, 2022)
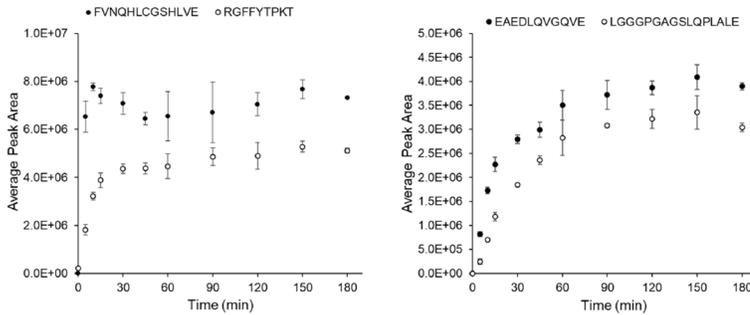
- Parallelism for Insulin and C-peptide--Horse serum is an adequate matrix for calibrators (Foulon, JMSACL, 2022)
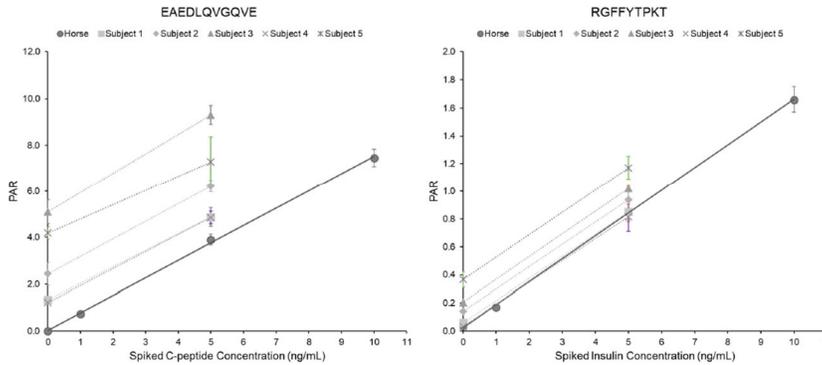
- Transferring the Method to Other Laboratories
  - Detailed standard operating procedure (Foulon, JMSACL, 2022, Owusu, JMSACL, 2021)

Owusu, et al., 2020
Supplemental Material

Standard Operating Procedure

Hoofnagle Laboratory
Department of Laboratory Medicine
University of Washington
Unofficial copy, Do Not Use For Patient Care

Principle

Human C-peptide is a 31-amino-acid polypeptide with a molecular mass of approximately 3020 Daltons. It originates in the β-cells of the pancreas and is released into the blood as a by-product of the enzymatic cleavage of the biologically inactive proinsulin to insulin. In this process, insulin and C-peptide are released from the prohormone and secreted into the portal circulation in equimolar concentrations. Since C-peptide is produced at the same rate as insulin, C-peptide is a useful marker of endogenous insulin secretion.

This method quantifies the amount of C-peptide in serum or plasma. After precipitation, resuspension and solid phase extraction, reduction and alkylation of the intact protein, the endoproteinase Glu-C is used to cleave peptide bonds at the carboxyl terminal side of glutamic acid residues. The digested peptide mix solution is then injected into the LC-MS/MS instrument. The peptides derived from intact C-peptide protein are quantified as the peak area ratio with the

  - Central Calibrators and Planned Plate Maps
 - Things were looking pretty good

Prelim data C-peptide | Prelim data Insulin
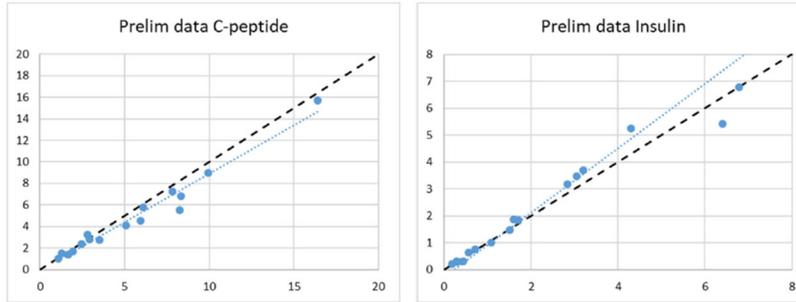
- o Then … intermittent to whole-plate failures
- o Modifications to the SOP in collaboration with TaMADOR
  - 1) Solid-phase extraction
    - Condition with methanol
    - Equilibrate with water
    - Add sample
    - Wash with 5% ammonium hydroxide
    - Wash with methanol
    - Elute in acidified methanol
  - 2) Root causes and fixes
    - Ammonium hydroxide is unstable
      1) Use fresh stock daily
      2) Keep sealed until use
      3) Repeater pipet instead of reagent boat
    - Vacuum manifold causing cracking
      1) Positive pressure SPE
      2) Ensure proper seal
  - 3) No more plate failures!
- Precision of the Measurement of C-peptide and Insulin: Interlaboratory comparison (Moradian, Clin Chem, 2024)

Interlaboratory Comparison for C-peptide — Median CV=13.4%
Interlaboratory Comparison for Insulin — Median CV=21.9%
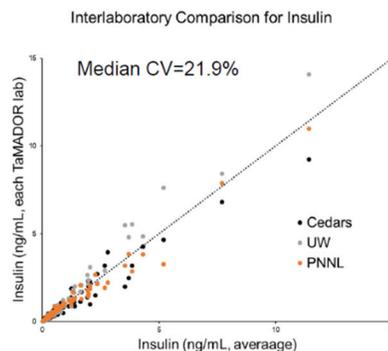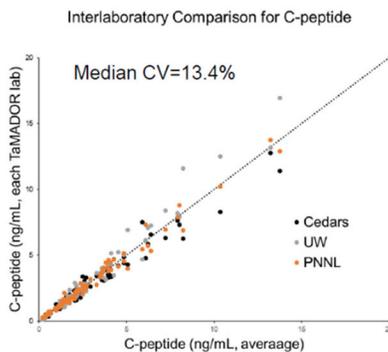
- Accuracy of the Measurement of C-peptide and Insulin: Method comparison with (candidate) reference methods (Moradian, Clin Chem, 2024)

C-peptide vs. Reference Method — y = 1.044x + 0.016, R² = 0.993
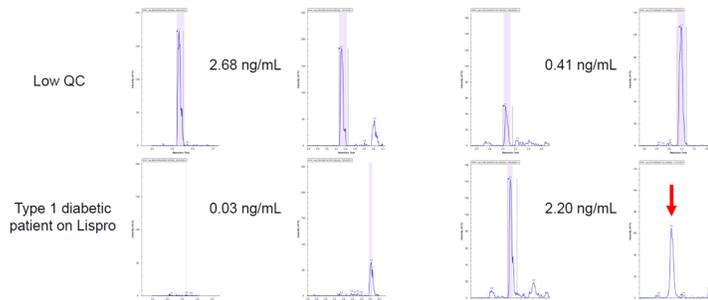TaMADOR vs. Reference Method — Mean Bias = 5.2%
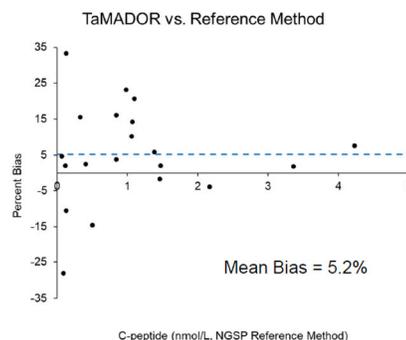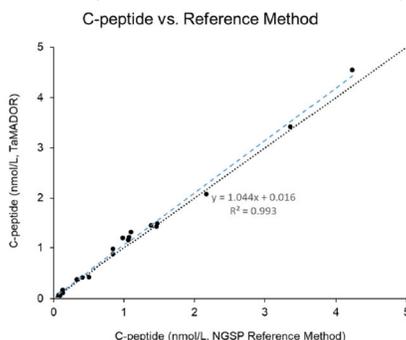
- Preanalytical Factors Affect Measurements: Comparison of C-peptide with insulin (Moradian, Clin Chem, 2024)
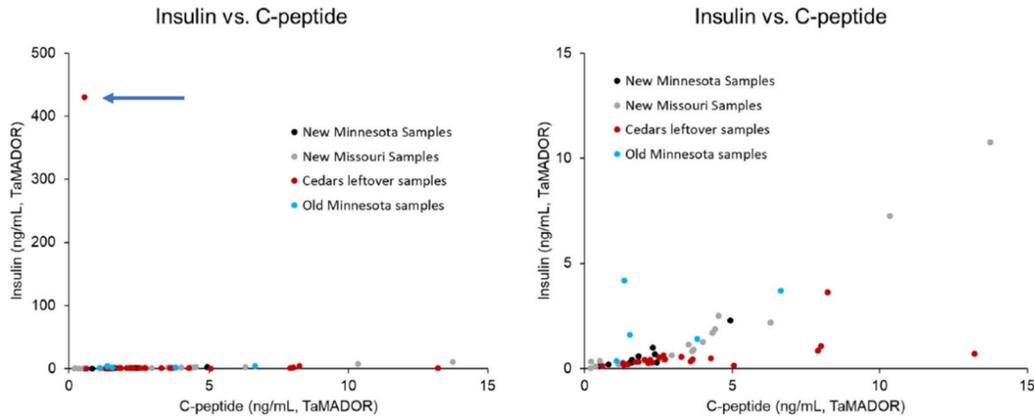
- Novel Method for C-peptide and Insulin: Application to clinical research (Gray, Diabetes, 2024)
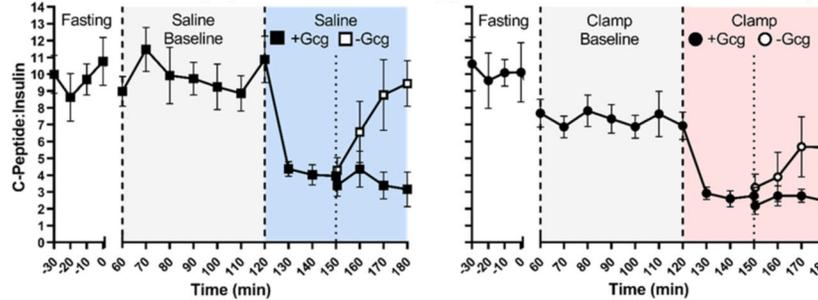
- Novel Method for C-peptide and Insulin: An interesting patient

- Novel Method for C-peptide--Adapted by NGSP: J. Mass Spectrometry Adv. Clin. Lab, 36 (2025), pp. 1-8
- Novel Method for Insulin
  - Used as the comparator for assessing cross-reactivity: Li, et al. Endocrine. 2025 Jan;87(1):79-84. doi: 10.1007/s12020-024-03970-6.
  - Used as the comparator for assessing standardization: Clin Chem Lab Med. 2025 Aug 14;63(12):2442-2446. doi: 10.1515/cclm-2025-0552. 2025.
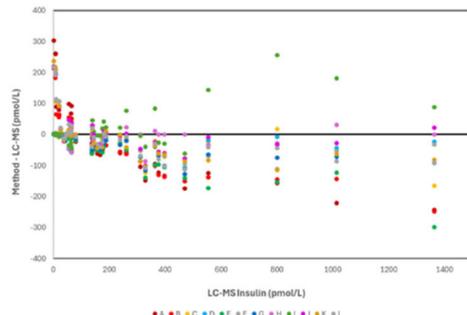
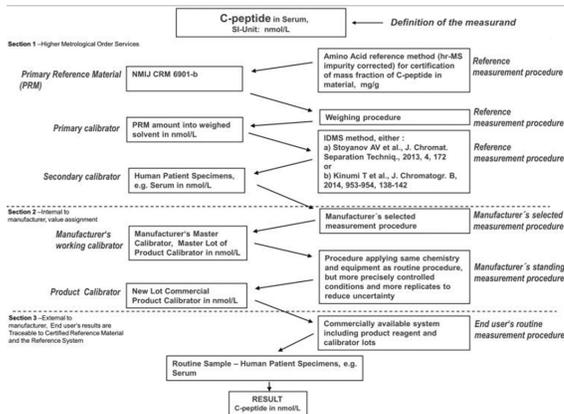**Figure 2:** Bias plot showing the individual differences for each sample vs. IDMS.

- A Multiplexed Assay for C-peptide and Insulin: Summary
  - GluC is a robust enzyme
    1) Short digestion time
    2) Multiplexable for small proteins
  - The assay is transferable
    1) 96-well plate format
    2) No antibodies needed
    3) Three labs, three different LC systems, three different mass specs
    4) Great precision
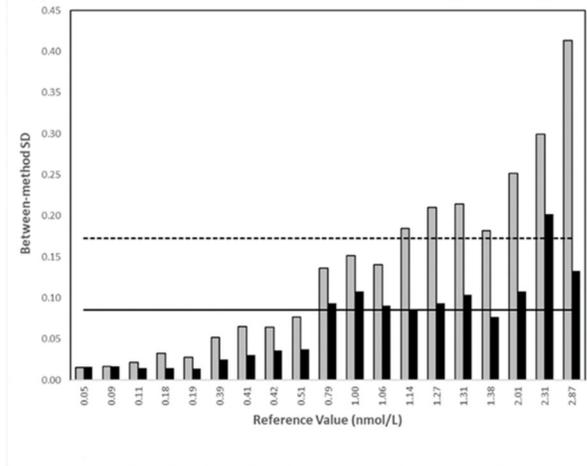 - Standardization is possible: Reference laboratories can help us be accurate

Discussion:

A.Hoofnagle noted that when they compared data obtained when the labs used the UW matrix-matched calibrators vs. the labs' own calibrators, the interlaboratory variability improved for C-peptide but less so for insulin. K. Kabytaev asked A. Hoofnagle if the University of Washington laboratory will be able to analyze the next set of comparison samples that will be prepared at the University of Missouri. A. Hoofnagle said the assay is still being used for several studies and they should be able to analyze the samples. K. Kabytaev noted that it can be difficult to transfer IDMS assays to other laboratories, especially when they are using different MS systems. The TaMADOR C-peptide comparison data look good, but insulin is more challenging, he asked A. Hoofnagle for suggestions regarding this. A. Hoofnagle said they like P800 tubes. Insulin is more challenging, the reference method being developed will be helpful for calibration but we will likely continue to see higher imprecision for the insulin assay compared to C-peptide. The pre-analytical variables can be overcome to some extent by using the P800 tube which has a lot of protease inhibitors and makes things more stable , they are expensive but worth it. K. Kabytaev asked about the use of a certified reference material for the insulin assay, A. Hoofnagle replied that their assay is calibrated with a reference material but it is in short supply, it is certified but not part of JCTLM. If a certified reference material does get listed with JCTLM they can use that to calibrate more accurately. G. Martos asked whether there was an equilibration step with labelled C-peptide before sample ACN precipitation, A. Hoofnagle responded that the equilibration step is short (~15 min), they do not do an extended equilibration, the recovery study they performed showed that this step was not necessary. K. Kabytaev asked whether the equilibration time is important for insulin, A. Hoofnagle replied that the harder and longer the agitation prior to centrifuging the more reliably insulin and C-peptide are dissociated from other proteins they may be bound to. They tested acid dissociation with C-peptide and insulin but obtained poor recoveries so they do not use it for these analytes. K. Kabytaev noted the importance of using the right tubes for the precipitation process, A. Hoofnagle agreed stating that they used low-binding tips, plates and tubes when important. This is especially true for low insulin concentrations.

3) C-Peptide/Insulin Update—Kuanysh Kabytaev
- Certified reference material
  - JCTLM-registered LC-MS reference method
  - Secondary reference material: pooled and single-donor serum samples
  - Comparisons: accuracy-based proficiency testing (ABGIC) and method comparison studies with commercial assay and LC-MS methods from academic labs (NMIJ Japan, QUEST, Albert Einstein College of Medicine NY, University of Washington)
- C-Peptide Traceability: Linking Patient Results to Reference Point

Figure: C-peptide in Serum, SI-Unit: nmol/L — traceability chain (Section 1 – Higher Metrological Order Services; Section 2 – Internal to manufacturer, value assignment; Section 3 – External to manufacturer, End user's results are Traceable to Certified Reference Material and the Reference System)

- Impact of Recalibration on Between-Assay Discrepancy (https://doi.org/10.1515/cclm-2024-1260)

- How Accurate Are Assays at Low Levels (< 0.2 nmol/L) before recalibration?

	Manufacturer's method		
Reference, nmol/L	min, nmol/L	max, nmol/L	mean, nmol/L
0.05	0.05	0.11	0.07
0.07	0.07	0.14	0.10
0.09	0.06	0.12	0.08
0.09	0.07	0.14	0.09
0.11	0.11	0.21	0.15
0.12	0.08	0.15	0.11
0.13	0.13	0.25	0.19
0.13	0.10	0.18	0.13
0.18	0.18	0.32	0.24
0.19	0.14	0.26	0.19

- ABGIC Survey for C-peptide
  - 2024B (Total 15 labs)

C-Peptide nmol/L METHOD	ABGIC-04							ABGIC-05							ABGIC-06						
	N	MEAN	SD	CV%	MEDIAN	MIN	MAX	N	MEAN	SD	CV%	MEDIAN	MIN	MAX	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
ALL METHODS	15	3.317	0.511	15.4	3.42	2.49	4.03	15	2.305	0.345	15.0	2.31	1.78	2.82	15	1.336	0.208	15.6	1.38	1.01	1.63
Roche cobas e600 series/E170	3	-	-	-	3.80	3.56	4.03	3	-	-	-	2.72	2.48	2.82	3	-	-	-	1.59	1.43	1.63
Roche cobas e801/e402	4	-	-	-	3.75	3.40	3.80	4	-	-	-	2.61	2.39	2.64	4	-	-	-	1.54	1.39	1.55
Reference Method**		2.430							1.710							1.110					

  - ABGIC Survey for C-peptide: 2025A (Total 21 labs)

C-Peptide nmol/L METHOD	ABGIC-01							ABGIC-02							ABGIC-03						
	N	MEAN	SD	CV%	MEDIAN	MIN	MAX	N	MEAN	SD	CV%	MEDIAN	MIN	MAX	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
ALL METHODS	21	1.328	0.200	15.1	1.40	0.95	1.56	20	4.576	0.705	15.4	4.81	3.40	5.44	21	2.826	0.403	14.3	2.96	2.08	3.24
Roche cobas e600 series/E170	5	-	-	-	1.47	1.22	1.54	5	-	-	-	5.13	4.65	5.29	5	-	-	-	3.20	2.94	3.23
Roche cobas e801/e402	5	-	-	-	1.50	1.45	1.56	5	-	-	-	5.07	4.88	5.44	5	-	-	-	3.18	3.00	3.24
Tosoh ST AIA-Pack	3	-	-	-	1.40	1.35	1.44	3	-	-	-	4.76	4.66	4.86	3	-	-	-	2.89	2.88	3.02
Reference Method**		0.91							3.10							2.03					

- Accuracy of LC-MS supplemental method (https://doi.org/10.1016/j.jmsacl.2025.02.001)
  - Accuracy of C-peptide measurements at low C-peptide concentrations.

- o Samples were prepared by spiking C-peptide CRM material into horse serum, and C-peptide levels were quantified using the proposed approach.
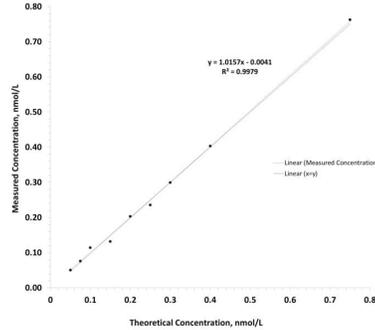
- • Why Measure Insulin Clinically
  - o Diagnose hypoglycemia causes (e.g. insulinoma, insulin overdose, drug-induced, post-bariatric)
  - o Differentiate insulin sources (endogenous vs exogenous)
  - o Guide treatment in post-bariatric hypoglycemia
- • Discordance among Insulin Assays (doi: 10.1515/cclm-2025-0552)
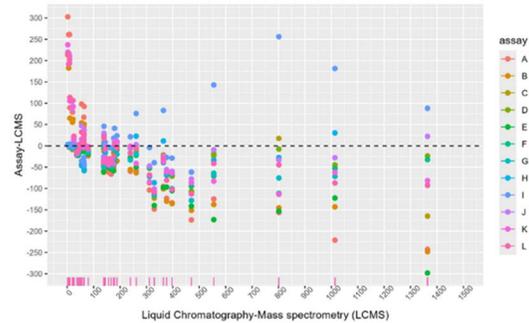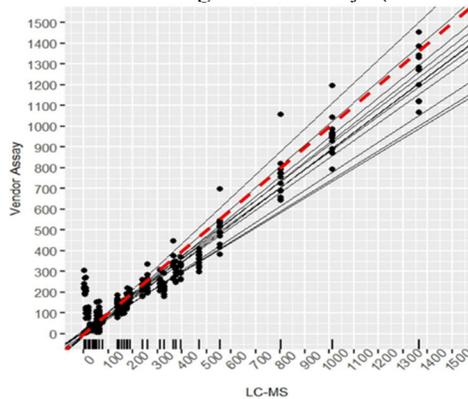
- • Insulin LC-MS method
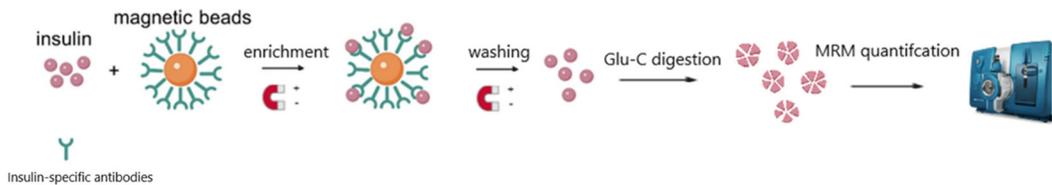
- • Summary
  - o C-peptide reference system in place (JCTLM-registered LC–MS method).
  - o Secondary reference materials available; new lots in preparation.
  - o Insulin LC–MS reference method under development.
  - o Open to collaboration to improve C-peptide and insulin testing
- •

Discussion:

Insulin method
A. Hoofnagle asked K. Kabytaev if he has found any benefit to using more than one antibody for the insulin method. K. Kabytaev responded that they use only one and have not tried using more than one. A. Hoofnagle said the concern is potential interference from insulin autoantibodies, using more than one antibody may help overcome this interference. K. Kabytaev said he will look into this approach. He noted that the next set of 40 samples will be more challenging to prepare, as they will be targeting insulin levels and wider C-peptide levels. R. Wielgosz asked A. Hoofnagle if he wants an insulin reference material that is ISO 15194 compliant, A. Hoofnagle said yes, they

would like to have a material that meets JCTLM requirements. K. Kabytaev asked R. Wielgosz if their group worked with a WHO-certified insulin material as well as a C-peptide material. R. Wielgosz responded that years ago when working on a C-peptide material they also worked on characterizing an insulin material as a research project at their institution. It was never published and there was never a reference material produced, but it that is a priority they can put it on the list to work on going forward. It would require collaboration.

Manufacturer feedback

K. Kabytaev asked attending manufacturer representatives for feedback. Regarding the next collection of 40 samples, some manufacturers previously asked for more samples and higher levels at the high end of the range (up to 10 nmol). Is this still needed or is the previous range (up to 4 nmol) sufficient? C. Vogl said that it would be good to span the concentration range, which for their assay goes up to 13 nmol, since spiked samples would need to be used commutability needs to be demonstrated. K. Kabytaev said these and even higher levels can be obtained by spiking. C. Vogl added that if these samples were to be used for re-calibration substantial sample volumes would be required, as they would have to run the samples a number of times on many different platforms. What are the goals in terms of the amounts and numbers of samples that will be prepared? K. Kabytaev noted that a need for 120 samples had been mentioned in previous meetings. He hoped that all manufacturers will participate in the next 40 sample comparison study in order to look at specificity, feasibility of using spiked materials and the performance at the low end of the range. When companies are ready to re-calibrate the study design will be different. J. Deeter noted that the 120 samples mentioned previously were a reference to the CLSI guideline. C. Vogl said that in addition it would be best to have a separate independent sample panel for a method comparison to verify the re-standardization, as has been done for Vitamin D standardization. Regarding sample volumes K. Kabytaev said that multiple vials of sample sets can be provided as needed. In the previous study some manufacturers ran samples in duplicate while others ran in singleton, for the next study manufacturers will be asked to run duplicates to obtain better statistical coverage, especially at the low end of the range. Dr. Murakami's group in Japan will be doing a similar study with our group. K. Kabytaev said 40 samples will be sent to the manufacturers to perform both C-peptide and insulin analyses, as was done in the previous study. Regarding the CAP surveys, A. Hoofnagle noted that most labs running C-peptide currently subscribe to the ING PT survey, which uses non-matrix matched material and peer-group grading. That survey is going to be replaced by the ABGIC survey in 2026, which will increase the number of labs participating in ABGIC from ~20 to >300. For this survey the UM-assigned values will be mentioned and cited as the target values, then over the next few years it will eventually move over to accuracy-based grading as was done for HbA1c. CAP is really pushing for this in the case of C-peptide, it will give us a lot more data to compare manufacturer results and cause labs to demand that manufacturers standardize their assays. Therefore it is a good time for manufacturers to re-calibrate, it is good that UM is providing a resource for them to do so. E. Schleicher if there is a timeframe for when the samples are sent out then the data are compiled and statistics completed. K. Kabytaev responded that the samples will be collected in early 2026, then UM will communicate with the different manufacturers regarding timelines with a goal of obtaining as much data and information as possible. They hope to obtain all of the data by mid-to-late 2026, but it takes some companies more time than others to complete the analyses. Hopefully the next study can include even more companies than were included in the previous study. JM Garcia asked about spiking the high level samples, is the conformational structure the same as for native C-peptide (are all isoforms clinically relevant)? K. Kabytaev replied that only a few samples will be spiked, as the very highest levels being requested are impossible to find in the patient population. For C-peptide conformational structure should not be an issue but the spiking of material will be validated, also we results will be obtained from different manufacturers and feedback will be provided. It is possible that the antibodies for some assays may be affected by the use of spiking material, so it will need to be tested. Hopefully re-calibration of assays can begin soon, as more data from clinical trials are coming out and C-peptide is now being considered as a clinical endpoint for T1 therapy, meaning more uniform results are required in terms of clinical thresholds. Standardized results will become more important for both clinical care and medical research.

K. Kabytaev thanked everyone for their attendance, and said those present should feel free to contact him if there are any questions or concerns. He noted that the next study is being planned, and that manufacturers would be receiving questionnaires concerning the characteristics of their assays and sample requirements. He hoped that all manufacturers would be willing to participate in the study.

Minutes prepared by Curt Rohlfing 01/29/2026.