Comparability of C-Peptide Measurements – Current Status and Clinical Relevance

OPEN ACCESS

Authors
Sebastian Hörber[1, 2, 3], Matthias Orth[4], Andreas Fritsche[2, 3, 5], Andreas Peter[1, 2, 3]

Affiliations
1 Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital Tübingen, Germany
2 Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Germany
3 German Center for Diabetes Research (DZD), München-Neuherberg, Germany
4 Institute of Laboratory Medicine, Vinzenz von Paul Kliniken gGmbH, Stuttgart, Germany
5 Department of Internal Medicine IV, Division of Endocrinology, Diabetology and Nephrology, University of Tübingen, Germany

Key words
Diabetes, Prediabetes, Immunoassay, Standardization, Harmonization

received 06.09.2022
revised 26.10.2022
accepted 11.11.2022
published online 11.01.2023

Bibliography
Exp Clin Endocrinol Diabetes 2023; 131: 173–178
DOI 10.1055/a-1998-6889
ISSN 0947-7349

Georg Thieme Verlag, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
Sebastian Hörber, MD
Hoppe-Seyler-Straße 3
72076 Tübingen
Germany
Tel.: +49-7071-2985664, Fax: +49-7071-294690
Sebastian.hoerber@med.uni-tuebingen.de

ABSTRACT

C-peptide is an increasingly used and established marker for beta cell function by assessing endogenous insulin secretion. Accurate and comparable C-peptide measurements are needed in clinical practice and research studies. For example, to calculate HOMA-indices, the C-peptide/glucose ratio, and the classification of recently published novel subgroups of diabetes and prediabetes have used C-peptide measurements. Although the process for standardization of C-peptide measurements is advanced, its full implementation is still missing; therefore, the current status of the comparability of C-peptide measurements using different immunoassays is unclear. Here we compared five widely used C-peptide immunoassays on different analyzers (Abbott ALINITY i, DiaSorin Liaison XL, Roche Cobas e411, Siemens Healthineers ADVIA Centaur XPT, and Immulite 2000 XPi) using serum samples covering the clinically relevant C-peptide concentration range. Although all investigated immunoassays are traceable to the international reference reagent for C-peptide (NIBSC code: 84/510), results of C-peptide measurements showed significant differences between analyzers in the entire concentration range, especially with increasing C-peptide concentrations. The mean bias was largest (36.6 %) between results of the immunoassays by Roche and Siemens Healthineers (ADVIA Centaur XPT), and both assays revealed large discrepancies compared to immunoassays by Abbott, DiaSorin, and Siemens Healthineers (Immulite 2000 XPi). In contrast, the three latter assays showed similar C-peptide results (mean bias: 2.3 % to 4.2 %). Consequently, C-peptide discrepancies might affect clinical diagnosis and the interpretation of study results. Therefore, there is an urgent need to implement and finalize the standardization process of C-peptide measurements to improve patient care and the comparability of research studies.

Introduction

C-peptide is a 31 amino acids polypeptide secreted by pancreatic beta cells into the circulation in equimolar amounts to insulin. In contrast to insulin, C-peptide exhibits a prolonged biological half-life; therefore, its plasma concentrations are higher compared to insulin [1]. In clinical routine, C-peptide measurements are used to assess endogenous insulin secretion, to distinguish between type 1, type 2, and other specific types of diabetes, and for differential diagnosis of fasting hypoglycemia [1–4]. C-peptide is also used for the calculation of HOMA (homeostatic model assessment) indices (e. g., HOMA-2B or HOMA-IR), i. e., for estimating insulin secretion and insulin resistance, especially in patients on insulin therapy. Using C-peptide measurements, these indices are necessary for the classification of novel subgroups in diabetes and prediabetes [5, 6]. C-peptide measurements are also useful for assessing the insulin secretion capacity and therapeutic consequences in patients with diabetes, as proposed by the recently published C-peptide/glucose ratio [7]. Furthermore, preserved C-peptide concentrations are associated with lower complication rates in type 1 diabetes; the measurements of C-peptide is used as the primary outcome for clinical trials of novel type 1 diabetes therapies [8–10].

To reliably evaluate circulating C-peptide concentrations in daily practice, clinical trials, and research studies, a prerequisite is that results of different C-peptide immunoassay measurements should be comparable, i. e., C-peptide immunoassays are standardized [11]. In general, the following assumptions are needed to achieve standardization of a measurand: the biomarker has a well-defined molecular composition, and measurement results are traceable to a primary reference material using a reference management system [12]. Since the molecular composition of C-peptide is well-defined and a traceability chain for the standardization process has been proposed, standardization of C-peptide immunoassays can be achieved [13]. During the last decades, much efforts was made to standardize C-peptide immunoassays; more than three decades ago, the first international reference reagent (IRR) for human C-peptide (NIBSC code: 84/510) was established, and reference methods for the reliable and accurate measurement of C-peptide concentrations were published [14–18].

Since to which extent this system has been implemented is not known, the aim of the study was to investigate the current status of the comparability of C-peptide immunoassays by comparing the results of C-peptide measurements of the most widely used immunoassays. Furthermore, the impact on clinical decisions and interpretation of clinical trials and research studies is elaborated here, and the current status and efforts of the C-peptide standardization process are discussed.

Materials and Methods

Study design and sample material

The study was conducted as part of a quality control measurement approach at the Institute for Clinical Chemistry and Pathobiochemistry of the University Hospital Tübingen, Germany. Serum samples from clinical routine were randomly selected, and each sample was divided into at least five identical aliquots and immediately stored at –80 °C. All aliquots were completely anonymized and labeled with ongoing codes. All procedures were in accordance with the declaration of Helsinki and its later amendments and have been approved by the local ethics committee (project number 113/2014BO1).

C-peptide immunoassays

For comparison of C-peptide immunoassays, five different immunoanalyzer platforms were used in the study (▶ **Table 1**). C-peptide measurements were performed using the ADVIA Centaur XPT and the Immulite 2000 XPi (both from Siemens Healthineers, Eschborn, Germany) and the Cobas e411 (Roche Diagnostics, Mannheim, Germany) in the central laboratory of the Institute for Clinical Chemistry and Pathobiochemistry of the University Hospital Tübingen. Measurements using the Liaison XL (Diasorin, Dietzenbach, Germany) and the ALINITY i (Abbott, Wiesbaden, Germany) were performed at the MVZ Labor Ludwigsburg, Germany and the Institute of Laboratory Medicine at the Vinzenz von Paul Kliniken Stuttgart, Germany, respectively. All measurements were performed between August 2021 and October 2021.

Statistical analysis

Results from C-peptide immunoassays were converted and reported in SI units (1 µg/L = 0.331 nmol/L). Method comparison was conducted using Passing-Bablok regression analysis and Bland-Altman plots. Mean biases obtained by Bland-Altman analysis were reported as % difference as follows: (result of method B – result of method A)/average vs. average. Analyses were performed, and figures were created using GraphPad Prism 9.1.2 software.

Results

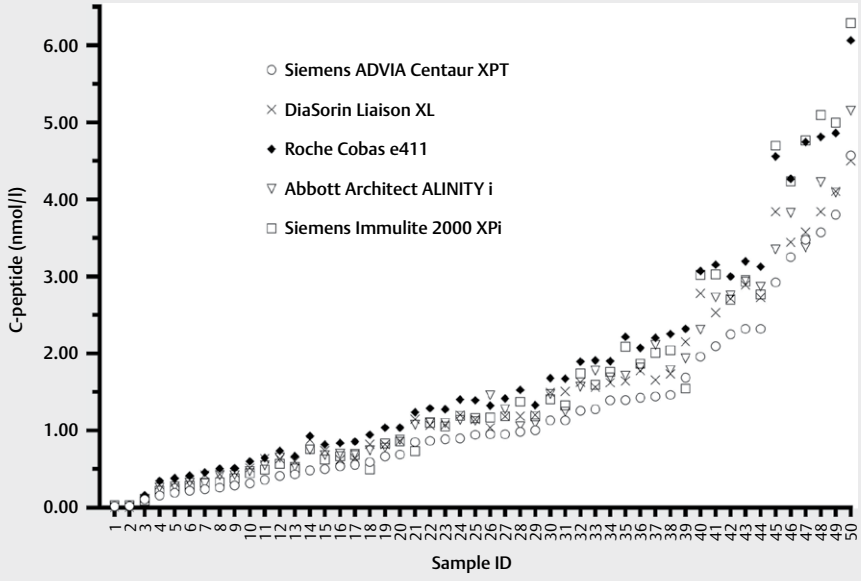
A total of 50 serum samples were used for the comparison of five commercially available and widely used C-peptide immunoassays (▶ **Table 1**). C-peptide measurement results ranged from 0.16–6.23 nmol/L depending on the investigated immunoassay. According to the German external quality assessment survey (report 05/2022 from INSTAND e.V.) the majority of all participating laboratories (94 %) are using one of these assays. Among them, the Roche C-peptide assay is the most widely used method (n = 101 of 191 participating laboratories). The C-peptide immunoassays from DiaSorin (n = 29), Abbott (n = 22), Siemens Healthineers ADVIA Centaur XPT/Atellica (n = 13), and Siemens Immulite (n = 14; former DPC Biermann) were used by a smaller number of laboratories in the same period. At the time of the study, all immunoassays were calibrated against the World Health Organization (WHO) international reference reagent (NIBSC code: 84/510).

▶ **Fig. 1** shows the results of C-peptide measurements using the aforementioned immunoanalyzers separated by single specimens. Substantial differences between immunoassays were observed in the entire concentration range, especially with increasing C-peptide concentrations. Since the majority of the participating laboratories in the external quality assessment use the Roche C-peptide immunoassay, Passing-Bablok analyses were conducted using the Roche C-peptide assay in comparison to the other C-peptide immunoassays. Linear associations were observed for all comparisons ($R^2 > 0.97$; see ▶ **Fig. 2**). Using Bland-Altman analysis to determine the mean bias between C-peptide immunoassays, the largest

▶ **Table 1** Analyzers and immunoassays used for C-peptide measurements in the study.

Platform/Analyzer	ALINITY i	ADVIA Centaur XPT	Cobas e411	Immulite 2000 XPi	Liaison XL
Manufacturer	Abbott	Siemens Healthineers	Roche Diagnostics	Siemens Healthineers	DiaSorin
Technology	CLIA	CLIA	ECLIA	CLIA	CLIA
Traceability of Calibrators	WHO IRR 84/510	WHO IRR 84/510	WHO IRR 84/510	WHO IRR 84/510	WHO IRR 84/510
Limit of Quantification/ Detection * (LoQ, nmol/L)#	0.03	0.02	0.05	0.03 *	0.03
Reference interval#, nmol/L	0.26–1.73	0.27–1.27	0.37–1.47	0.30–2.35	0.26–1.39

according to the manufacturer. Abbreviations: CLIA: chemiluminescence immunoassay; ECLIA: electrochemiluminescence immunoassay; IRR: international reference reagent; WHO: World Health Organization



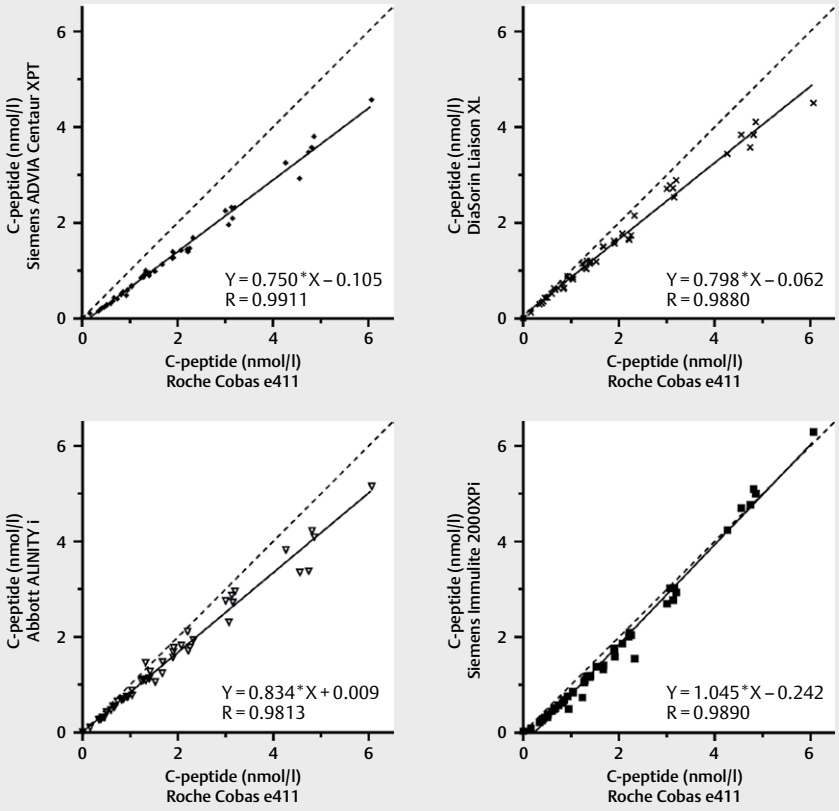
▶ **Fig. 1** Comparison of C-peptide measurements. Shown are results of C-peptide measurements (in nmol/L) separated by specimens using five widely used immunoassays.

difference (ADVIA Centaur XPT – Roche Cobas e411: mean bias –36.6 %; ▶ **Table 2**) was observed between C-peptide assays from Siemens Healthineers (ADVIA Centaur XPT) and Roche. The Siemens Healthineers ADVIA Centaur XPT assay also revealed lower C-peptide concentrations in comparison to the assays from Abbott (-20.3 %), DiaSorin (19.1 %), and Siemens Healthineers (Immulite; – 24.4 %). The Roche Cobas e411 assay showed higher C-peptide concentrations compared to Abbott (16.3 %), DiaSorin (18.6 %), and Siemens Healthineers Immulite (13.3 %). C-peptide assays by Abbott, DiaSorin, and Siemens (Immulite) exhibited a similar performance (mean biases < 5 %).

Discussion

In the present study, the current status of the comparability of the most widely used C-peptide immunoassays was compared. Substantial differences were found between different C-peptide immunoassays suggesting that the results of C-peptide measurements cannot be used interchangeably. Of note, assay discrepancies might directly affect the interpretation of C-peptide results and, therefore, impact clinical diagnosis and the evaluation of clinical trials and research studies.

The study investigated five widely used and commercially available C-peptide immunoassays using clinical samples covering the clinically relevant concentration range. Method comparison showed a linear association among C-peptide immunoassays of all manufacturers. However, focusing on the agreement of different immunoassays, C-peptide results measured by immunoassays from Siemens Healthineers (ADVIA Centaur XPT) and Roche (Cobas e411) showed large discrepancies in the low as well as high concentration range. Results obtained by C-peptide immunoassays from Abbott (ALINITY i), DiaSorin (Liason XL), and Siemens Healthineers (Immulite 2000 XPi) were similar. There are only a few reports of studies comparing the results of different C-peptide immunoassays. In line with the presented results, a study by Wiedmeyer showed substantial between-laboratory variabilities for



▶ **Fig. 2** Regression analysis of C-peptide immunoassays. Passing-Bablok regression analyses were conducted using the measurement results of the Roche C-peptide immunoassay in comparison to the C-peptide measurement results by Abbott, DiaSorin and Siemens Healthineers.

▶ **Table 2** Comparison of C-peptide immunoassays. Shown are the mean biases obtained by Bland-Altman analysis. Results are expressed in percentage and read as follows (example): C-peptide concentrations obtained using the Abbott ALINITY i immunoassay are higher (mean bias: +20.3 %) compared to the average concentrations of the Siemens ADVIA Centaur XPT and the Abbott ALINITY i C-peptide immunoassays.

	Abbott ALINITY I vs.	Siemens ADVIA Centaur XPT vs.	Roche Cobas e411 vs.	Siemens Immulite 2000 XPi vs.	DiaSorin Liaison XL vs.
Abbott ALINITY i	–	−20.3 %	+16.3 %	+3.8 %	−2.3 %
Siemens ADVIA Centaur XPT	+20.3 %	–	+36.6 %	+24.4 %	+19.1 %
Roche Cobas e411	−16.3 %	−36.6 %	–	−13.3	−18.6 %
Siemens Immulite 2000 XPi	−3.8 %	−24.4 %	+13.3 %	-	−4.2 %
DiaSorin Liaison XL	+2.3 %	– 19.1 %	+18.6 %	+4.2 %	-

C-peptide measurements [19]. This study was performed about fifteen years ago and demonstrated that normalization of C-peptide results using commutable sample calibrators modestly reduces assay discrepancies. However, this approach is ineffective for comparison of clinical trials, indicating the need for timely standardization of C-peptide measurements. In a recent study by Zhou et al., C-peptide immunoassays from Abbott, Roche, and Siemens Healthineers showed similar discrepancies [20]. They used pooled serum samples and compared C-peptide results between 94 laboratories in China. The analytical performance of the investigated assays was satisfactory, but they also found substantial differences between immunoassays and concluded, in line with the present results, that still much effort has to be done to standardize C-peptide measurements.

All C-peptide immunoassays investigated in the present study were calibrated against the first international reference reagent (IRR, NIBSC code: 84/510) at the time of the study. This standard was established more than 30 years ago and has been used by the manufacturers for direct calibration of their immunoassays. Since 2017, the first international WHO C-peptide standard (NIBSC code 13/146) is available; it was established using more accurate methods compared to the preparation of the IRR [21]. The IRR 84/510, as well as the WHO standard 13/146 contain purified human C-peptide. In an international comparison study, both standards showed reasonable agreement between laboratories [21]. The stocks of the IRR 84/510 are exhausted; thus, the WHO standard 13/146 will replace the IRR. However, the reference management system for the international WHO standard 13/146 using the primary refer-

ence material directly for calibration of the immunoassay is the same as for the IRR 84/510, which was demonstrated to be ineffective in improving comparability between C-peptide immunoassays [19]. In parallel to these efforts, another primary reference material has been established by the National Metrology Institute of Japan (NMIJ; CRM 6901-b) recommended by the international C-peptide standardization committee as primary reference material for a recently proposed and modified reference management system [13, 22]. This reference management system uses commutable matrix-based reference materials for the calibration of immunoassays by manufacturers. The use of matrix-appropriate secondary reference materials (frozen serum samples) was demonstrated to substantially improve measurement results among methods [13]. However, these ongoing parallel efforts lead to confusion among the manufacturers and clinical laboratories. Furthermore, from the perspective of a manufacturer, regulatory issues regarding the re-calibration of C-peptide immunoassays have to be considered that can vary significantly among countries [23]. To improve efforts by the manufacturers and clinical laboratories for the standardization process, the incorporation of standardization requirements for C-peptide immunoassays in clinical guidelines may be of great importance.

Recognizing the increasing importance of C-peptide measurements, the presented discrepancies among C-peptide immunoassays impact clinical diagnosis and comparability of study results. The recently proposed C-peptide/glucose ratio (CGR; both in the fasting state) as a marker for the insulin secretory capacity was established from a large cohort of patients with newly diagnosed or known type 2 diabetes [7]. In contrast to the complex determination of HOMA indices, the CGR can be easily obtained and is, therefore, a useful parameter in clinical practice. The ratio is suggested to guide treatment decisions for patients with type 2 diabetes, especially to evaluate if there is a need for insulin treatment. For the calculation of these cut-offs, the C-peptide immunoassay from Siemens Healthineers (ADVIA Centaur XPT) was used. Considering the present study results, it can be assumed that there will not be entirely negligible differences in the calculated CGR when using different C-peptide immunoassays. The largest difference was found between the Roche Cobas immunoassay and the ADVIA Centaur XPT, potentially affecting therapy decisions based on the CGR cut-offs. Whether these differences actually affect the outcome of diabetes patients have to be investigated in further studies. Moreover, the proposed novel diabetes subgroups by Ahlqvist et al. and also the prediabetes subphenotypes proposed by Wagner et al. use HOMA-indices for the subgroup stratification [5, 6]. Since C-peptide measurements were used for HOMA calculations, the present results indicate that the choice of the C-peptide immunoassay might affect the subgroup stratification. There are several variables included in the stratification of the subgroups and, therefore, the impact of C-peptide differences for a single patient is unclear. However, the use of different C-peptide immunoassays at different study sites clearly affects the comparability of study results. Furthermore, C-peptide is also an important parameter in type 1 diabetes. It is used in clinical trials as endpoint in the immunomodulatory therapies for type 1 diabetes and also as a prognostic marker for type 1 diabetes-related complications [8–10]. For example, the response to teplizumab, an anti-CD3 antibody that can delay progression to type 1 diabetes in high-risk subjects, is greater in subjects with lower C-peptide concentrations during an oral glucose tolerance test compared to subjects with higher concentrations [8]. In the Scottish Diabetes Research Network Type 1 Bioresource (SDRN-T1BIO), cohort it was demonstrated that residual C-peptide concentrations could improve clinical outcomes in type 1 diabetes, and also in the Diabetes Control and Complications Trial (DCCT), measurable C-peptide concentration was associated with beneficial clinical outcomes [9, 10]. Therefore, reliable and comparable C-peptide concentrations, especially in the low concentration range in this context, are a prerequisite.

In light of this variety of important indications for C-peptide measurements, the C-peptide standardization process needs to be implemented and finalized in the near future. Until this approach is reached, it is important that C-peptide measurements are conducted using the same immunoassay. In multi-center studies, C-peptide measurements should be performed at one study site using the same immunoassay. In clinical practice, physicians should be aware of assay discrepancies when comparing C-peptide results of the patients, measured at different laboratories. To monitor the next steps of the standardization process and verify the current status of the comparability participation in external quality assessment programs is recommended for laboratories [13, 24].

In conclusion, this study demonstrates that results of different C-peptide immunoassays do not always agree, and assay discrepancies should be considered when interpreting C-peptide concentrations in the context of clinical decisions and clinical trials. To overcome these discrepancies, the proposed standardization process should be urgently implemented to improve patient care and the comparability of research studies.

Acknowledgements

We thank M. Siegle (MVZ Labor Ludwigsburg) for performing the C-peptide measurements using the DiaSorin Liaison XL. Reagents for C-peptide measurements using the Liaison XL were kindly provided by DiaSorin.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Jones AG, Hattersley AT.. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med 2013; 30: 803–817. doi:10.1111/dme.12159

[2] Palmer JP, Fleming GA, Greenbaum CJ et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: Report of an ADA workshop, 21-22 October 2001. Diabetes 2004; 53: 250–264

[3] Arzamendi AE, Rajamani U, Jialal I.. Pseudoinsulinoma in a white man with autoimmune hypoglycemia due to anti-insulin antibodies: value of the free C-Peptide assay. Am J Clin Pathol 2014; 142: 689–693. doi:10.1309/AJCPX56JQBJHUBGJ

[4] Maddaloni E, Bolli GB, Frier BM et al. C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective. Diabetes Obes Metab 2022; 24: 1912–1926. doi:10.1111/dom.14785

[5] Ahlqvist E, Storm P, Karajamaki A et al. Novel subgroups of adult-onset diabetes and their association with outcomes: A data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018; 6: 361–369. doi:10.1016/S2213-8587(18)30051-2

[6] Wagner R, Heni M, Tabak AG et al. Pathophysiology-based subphenotyping of individuals at elevated risk for type 2 diabetes. Nat Med 2021; 27: 49–57. doi:10.1038/s41591-020-1116-9

[7] Fritsche A, Heni M, Peter A et al. Considering insulin secretory capacity as measured by a fasting C-peptide/glucose ratio in selecting glucose-lowering medications. Exp Clin Endocrinol Diabetes 2022; 130: 200–204. doi:10.1055/a-1242-9809

[8] 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. doi:10.1056/NEJMoa1902226

[9] Jeyam A, Colhoun H, McGurnaghan S et al. Clinical impact of residual C-peptide secretion in type 1 diabetes on glycemia and microvascular complications. Diabetes Care 2021; 44: 390–398. doi:10.2337/dc20-0567

[10] Lachin JM, McGee P, Palmer JP et al. Impact of C-peptide preservation on metabolic and clinical outcomes in the Diabetes Control and Complications Trial. Diabetes 2014; 63: 739–748. doi:10.2337/db13-0881

[11] Horber S, Achenbach P, Schleicher E et al. Harmonization of immunoassays for biomarkers in diabetes mellitus. Biotechnol Adv 2020; 39: 107359. doi:10.1016/j.biotechadv.2019.02.015

[12] International Organization for Standardization. In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples. In. ISO 17511:2020: International Organization for Standardization; 2020

[13] Little RR, Wielgosz RI, Josephs R et al. Implementing a reference measurement system for C-peptide: Successes and lessons learned. Clin Chem 2017; 63: 1447–1456. doi:10.1373/clinchem.2016.269274

[14] Bristow AF, Das RE.. WHO international reference reagents for human proinsulin and human insulin C-peptide. J Biol Stand 1988; 16: 179–186

[15] Kinumi T, Mizuno R, Takatsu A.. Quantification of serum C-peptide by isotope-dilution liquid chromatography-tandem mass spectrometry: Enhanced detection using chemical modification and immunoaffinity purification. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 953–954: 138–142. doi:10.1016/j.jchromb.2014.02.019

[16] Stoyanov AV, Rogatsky E, Stein D et al. Isotope dilution assay in peptide quantification: The challenge of microheterogeneity of internal standard. Proteomics Clin Appl 2013; 7: 825–828. doi:10.1002/prca.201200130

[17] Stoyanov AV, Rohlfing CL, Connolly S et al. Use of cation exchange chromatography for human C-peptide isotope dilution - mass spectrometric assay. J Chromatogr A 2011; 1218: 9244–9249. doi:10.1016/j.chroma.2011.10.080

[18] Taylor SW, Clarke NJ, Chen Z et al. A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide. Clin Chim Acta 2016; 455: 202–208. doi:10.1016/j.cca.2016.01.019

[19] Wiedmeyer HM, Polonsky KS, Myers GL et al. International comparison of C-peptide measurements. Clin Chem 2007; 53: 784–787. doi:10.1373/clinchem.2006.081570

[20] Zhou W, Deng Y, Zhao H et al. Current status of serum insulin and C-peptide measurement in clinical laboratories: Experience from 94 laboratories in China. Ann Lab Med 2022; 42: 428–437. doi:10.3343/alm.2022.42.4.428

[21] Moore M, Dougall T, Ferguson J et al. Preparation, calibration and evaluation of the First International Standard for human C-peptide. Clin Chem Lab Med 2017; 55: 1224–1233. doi:10.1515/cclm-2017-0029

[22] Kinumi T, Goto M, Eyama S et al. Development of SI-traceable C-peptide certified reference material NMIJ CRM 6901-a using isotope-dilution mass spectrometry-based amino acid analyses. Anal Bioanal Chem 2012; 404: 13–21. doi:10.1007/s00216-012-6097-1

[23] The C-Peptide Standardization Commitee. 2021 C-Peptide Standardization Manufacturer Meeting Minutes. In http://cpeptide.org/assets/cpeptide2021.pdf

[24] Hörber S, Kaiser P, Achenbach P et al. Neue klassifikation des diabetes mellitus – Anforderungen an Labormessgrößen. Diabetologie und Stoffwechsel 2021; 16: 63–69