results provide a harmonized terminology that addresses the shortcomings of currently used terms.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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Biological Variation of Hemoglobin A1c: Consequences for Diagnosing Diabetes Mellitus

To the Editor:

For optimal monitoring and diagnosing of patients with diabetes by use of glycated hemoglobin (Hb A1c) measurements, the analytical CV (CVa) of the Hb A1c assay and the within-person biological variation (CVwp) are of relevance. CVwp refers to an inherent biological variation around an individual patient’s set point of a biological parameter. Several studies have been published assessing the CVwp of Hb A1c (1–3). However, none of those studies used >1 Hb A1c method to determine the CVwp. The aim of this study was to apply 4 different IFCC and National Glycohemoglobin Standardization Program (NGSP) certified secondary reference measurement procedures (SRMPs) using different assay principles and calibrated in SI units (mmol/mol) and Diabetes Control and Complications Trial (DCCT) units (% Hb A1c) to see whether there were differences in the CVwp obtained. In addition, we addressed the consequences for diagnosing diabetes mellitus of the CVwp found.

We recruited 21 presumed healthy hospital employees to participate in the study (11 men and 10 women). Five K3EDTA-treated whole blood samples were collected from each individual every 2 weeks for 2 months. On collection, aliquots of each sample were immediately stored at −80 °C. Full analysis was performed at the end of the 2-month collection period. The samples were analyzed in a single run in duplicate using the following 4 SRMPs:

• Tina-quant Gen.2 HbA1c on Integra 800, immunoassay, IFCC and NGSP certified (Trinity Diagnostics);

• Premier Hb9210, boronate affinity chromatography HPLC, at the time not yet officially certified (Trinity Biotech);

• Tosoh G8, Cation-Exchange HPLC, IFCC certified (Tosoh Bioscience); and

• Ultra², boronate affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech).

All 4 SRMPs have documented good results in the IFCC and NGSP monitoring programs [CV <3.0% in SI units, <2.0% in DCCT units, no bias or a very small bias (±1 mmol/mol) compared to the IFCC primary reference measurement procedure (PRMP)] and were calibrated using the IFCC secondary reference material with assigned IFCC and derived DCCT values.

The data were analyzed using a 2-level nested ANOVA model.

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Nonstandard abbreviations: Hb A1c, glycated hemoglobin; CVa, analytical CV; CVwp, within-person biological variation; NGSP, National Glycohemoglobin Standardization Program; SRMP, secondary reference measurement procedure; DCCT, Diabetes Control and Complications Trial; PRMP, primary reference measurement procedure; CVbp, between-person biological variation.
This analysis model assumes homogeneity, normality, and independence when estimating CIs. The homogeneity of analytical imprecision was confirmed using Cochran test, and within-person variation was confirmed using Bartlett test. Homogeneity was achieved by removing 2 individuals from the data set (P = 0.006, P value adjusted for multiple testing). One individual was removed from the data set because of a clear linear increase in Hb A1c values during the 2-month testing period (i.e., dependence of data), and the other was removed from the data set because of increased Hb A1c values (previously undiagnosed diabetes). Normality was checked using Shapiro–Wilk test (P = 0.006) for both within-person and analytical components for the 4 methods. The index of individuality was calculated as CVwp/between-person biological variation (CVbp).

The mean CVwp of 19 healthy individuals using 4 SRMP Hb A1c methods was 1.3% in SI units (range 0.8% to 1.7%) and 0.8% in DCCT units (range 0.5% to 1.0%), depending on the Hb A1c method used (Table 1). The CVwp measured with the Ultra2 was significantly different (95% CI CVwp in SI units: 0.4–1.2) from the Tosoh G8 (1.4–2.0) but not from Roche Tina-quant Gen.2 (0.9–1.8) or the Premier Hb9210 (1.1–1.6).

All CVs calculated in DCCT units were much lower compared with CVs calculated in SI units (Table 1) owing to the lower specificity of the NGSP PRMP. The master equation for converting SI units to DCCT units is: DCCT = (0.0915 × IFCC) + 2.15, where the positive y-intercept value reflects the non-specificity of the NGSP PRMP method. When Hb A1c measured with the IFCC PRMP is zero, the NGSP PRMP method still measures “something” (low specificity), and this nonspecificity is added to the results, making the results in DCCT units higher. Because the SD will be the same in the 2 situations, the CV will be lower when calculated in DCCT units. The NGSP PRMP measures Hb A1c on the interval scale because of the positive y-intercept, whereas the IFCC PRMP measures on the ratio scale (4). In principle, CVs should not be calculated for constituents measured on the interval scale. In previous studies, CVwp in DCCT and SI units have been used interchangeably and compared with each other (2, 3, 5).

The Premier Hb9210 and the Ultra2 use the same assay principle measuring the same measurand, and therefore the difference in CVwp measured with the Tosoh G8 and the Ultra2 cannot be explained by measuring different measurand, since the CVwp measured with the Tosoh G8 and the Premier Hb9210 did not significantly differ from each other. As shown in Table 1, the CVbp was large (Table 1) and Hb A1c had a marked individuality. The index of individuality varied between 0.11 and 0.22.

The mean Hb A1c of the healthy individuals ranged from 28 mmol/mol (4.7% DCCT) to 39 mmol/mol (5.7% DCCT). With the publication of the WHO and American Diabetes Association guidelines advocating the use of Hb A1c for the diagnosis of diabetes, there have been major shifts in the role of Hb A1c testing. The low in-

<table>
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<tr>
<th>Table 1. CVa, CVwp, and CVbp of Hb A1c in healthy individuals.(^a)</th>
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<tbody>
<tr>
<td><strong>Grand mean, %</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Ultra2</td>
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<td>mmol/mol</td>
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\(^a\) Data are SI units (mmol/mol) and DCCT units (%) (95% CI).

\(^b\) and \(^c\) are significantly different from each other.
change in Hb A1c may indicate a small change in Hb A1c, may indicate a clinically significant change, e.g., development of diabetes in a patient, even if the difference between the 2 results does not cross the threshold. The CVwp of stable diabetic patients should especially be used for monitoring purposes. However, as shown by Carlsen et al. (3), the CVwp of stable diabetic patients does not differ significantly from that of healthy individuals, and therefore it is probable that the findings in this study will also refer to stable diabetes patients.

In conclusion, the within-person biological variation of Hb A1c in healthy individuals is very low compared with the between-person biological variation of Hb A1c, affirming the absolute individuality of Hb A1c. The data also suggest that population-based reference limits and fixed cutoff values should be more closely examined for the diagnosis of diabetes. CVs in DCCT units are lower than CVs in SI units and therefore cannot be directly compared.

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Carcinoembryonic Antigen Monitoring to Detect Recurrence of Colorectal Cancer: How Should We Interpret the Test Results?

To the Editor:

It is routine clinical practice, supported by national guidelines in both North America and Europe, to measure blood carcinoembryonic antigen (CEA)1 to detect recurrence of colorectal cancer during follow-up after primary treatment. Blood CEA is usually measured every 3–6 months, and patients with a CEA concentration above an absolute threshold (5 µg/L according to American Society of Clinical Oncology guidelines) are investigated further by radiological imaging. However, the evidence underpinning both guidelines and routine practice is weak.

We recently reported the interim results of the Follow-up After Colorectal Surgery (FACS) trial, a clinical trial comparing different types of posttreatment follow-up in 1200 patients with colorectal cancer (1). This trial confirmed that measuring CEA is an effective way of detecting recur-

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1 Nonstandard abbreviations: CEA, carcinoembryonic antigen; FACS, Follow-up After Colorectal Surgery; AUC, area under the curve.

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