for the Afinion method (between 1.2% and 2.7%) and the in2it method (between 2.4% and 3.0%). For the Afinion method, imprecision was slightly better for the QC material than for whole blood. The whole blood estimate may better reflect the variation in patient whole blood results for the Afinion method.

The 95% confidence limits for the differences between the methods and the NGSP SRLs are also shown in Table 1. In laboratory A, both A1cNow lots passed the NGSP certification criteria, whereas both lots failed in laboratory B. For the Afinion method, one of the lots passed in laboratory A, and the second lot failed. In laboratory B, both lots passed. Both in2it lots passed NGSP certification.

For the A1cNow method, there was no significant difference between pairs of reagent lots within each laboratory (P = 0.72, and P = 0.49 in laboratories A and B, respectively). The results for the 2 lots in laboratory A, however, were significantly different from those for the 2 lots in laboratory B (P < 0.0001). The Afinion method showed a significant difference between lots in laboratory A (P = 0.0002), a marginal but insignificant difference between lots in laboratory B (P = 0.05), and a significant difference in lots between the 2 laboratories (P < 0.0001). For the in2it method, there was a very small but significant difference (P = 0.02) between the 2 lots of reagent in a single laboratory.

Although the ideal analytical goal for the imprecision of Hb A1c methods is ≤2%, CVs of ≤3% are certainly reasonable. In the present study, the Afinion imprecision was similar to previous results, with CVs of <3%. The A1cNow CVs were considerably greater than 3% and were therefore considered unacceptable. The total CVs for the in2it method were improved over those of the original evaluation and were acceptable in the current evaluation. Lot-to-lot variation for the A1cNow and Afinion methods is a concern. Two of 4 A1cNow lots and 1 Afinion lot did not pass NGSP certification.

It is important to consider clinical needs when selecting Hb A1c assay methods, including POC methods. Clinicians must recognize that although POC Hb A1c measurement offers convenience in some clinical settings, the performance of some POC methods may not be sufficient to meet clinical needs.

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Gross Overestimation of Total Allowable Error Based on Biological Variation

To the Editor:

Westgard was the first to introduce the concept of total error (TE) in 1974. Analytical imprecision and bias [i.e., systematic error (SE)] were combined into a single measure of the uncertainty of a test result:

\[ \text{TE} = \text{Bias} + 1.65 \times \text{Imprecision} \]

The factor 1.65 implies that 95% of the results (1-sided) will fall within the TE limit, given a gaussian distribution.

\[^{1}\text{Nonstandard abbreviations: TE, total error; SE, systematic error; TEa, total allowable error; CVa, interindividual imprecision; CVw, intranidividual imprecision; Cva, analytical imprecision.} \]
When the bias and imprecision are known, the uncertainty of the result can be calculated. When the maximum allowable total error (TEa) is known (e.g., on the basis of clinical needs or biological variation), the maximum allowable bias and imprecision can be calculated. The expression used in general for calculating TEa is:

$$\text{TEa} = \text{Allowable bias} + 1.65 \times \text{Imprecision}$$

where the allowable bias is $$0.25 \times (\text{CVw}^2 + \text{CVb}^2)^{1/2}$$, the allowable imprecision is $$0.5 \times \text{CVw}$$, CVb is the interindividual imprecision, and CVw is the intraindividual imprecision.

Values for CVb, CVw, and TEa are easily available (1), but where does this expression for TEa come from?

The allowable imprecision has been derived with different methods (2). As Stöckl et al. (2) stated, "A striking feature is the fact that all of the individual approaches described above [in Stockel et al.'s text] recommend numbers for analytical standard deviation near or equal to 0.5 times the biological standard deviation." This maximum analytical imprecision can easily be shown to add 12% to the biological variation.

The bias in the TEa expression was derived by Gowans et al. (3). The analytical goals were calculated for the successful transfer of reference intervals between laboratories. A maximum of 4.6% of a normal population outside a reference limit (as opposed to 2.5% with zero bias and imprecision) was considered the maximum acceptable level on the basis of the IFCC guideline on the calculation of reference values with $$n = 120$$ (4). The maximum bias in the expression is valid only in cases when the imprecision equals zero.

For use in external quality control, separate quality goals have been applied:

$$\text{Imprecision} < 0.5 \times \text{CVw} \text{, and}$$

$$\text{Bias} < 0.25 \times (\text{CVw}^2 + \text{CVb}^2)^{1/2}.$$  

Fraser and Hyltoft Petersen were the first—to my knowledge—to combine these 2 criteria in a single expression (5). This expression was a quality goal and was meant for use in External Quality Assessment Service reports. For this expression, however, no theoretical basis was presented. Fraser and Hyltoft Petersen indicated, "However, when only a single determination of each survey material is used or allowed, the 95% acceptance range (for total error) for each laboratory from the target value is:$$\pm 1.65 (0.5 \times \text{CVw}) + 0.25 \times (\text{CVw}^2 + \text{CVb}^2)^{1/2}$$ [published version in (5): $$\pm [1.65 (\frac{1}{2}\text{CVl}) + \frac{1}{2}(\text{CVl}^2 + \text{CVw}^2)^{1/2}]$$].

As an example, the calculations for creatine kinase displayed in Fig. 1 are as follows (1): CVw = 22.8%, CVb = 40.0%, the maximum bias (SE) is $$0.25 \times (\text{CVw}^2 + \text{CVb}^2)^{1/2} = 11.5\%$$, and the maximum analytical imprecision (CVa) is $$0.5 \times \text{CVw} = 11.4\%$$.

As Gowans et al. showed (3), the maximum allowable bias and imprecision are interrelated, and they can be described in the curve presented in Fig. 1 for CK (the CVa–SE curve). The values for maximum bias and imprecision calculated above can be found in tables (1) and are the extremes in the CVa–SE curve.

TEa can easily be calculated from each point of the SE–CVa curve (Fig. 1, TEa) by using the Westgard expression combining imprecision and bias:

$$\text{TEa}_1 = \text{SE} + 1.65 \times \text{CVa}.$$  

The maximum value of TEa in the curve (determined by inspection) is 18.9% (TEa1). This value is very close to 1.65 \times \text{CVa}(\text{max}). In
the conventional model, 2 maximum values for bias and imprecision, at the extremes of the curve, are added together to give the TEa estimate. This estimated TEa is a fixed number: TEa = 1.65 × (0.5 × CVw) + 0.25 × (CVw² + CVb)³/² = 30.3%.

Comparing 18.9% with 30.3%, we see that TEa is overestimated in the latter model. For different purposes, the maximum allowable imprecision and bias have been derived separately from data on biological variation. To combine these maximum values into a single expression has no theoretical basis and leads to gross overestimation of TEa.

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Using Nonfasting Lipids—Hemodilution or Convenience?

To the Editor:

Langsted and Nordestgaard, of the Copenhagen General Population Study (CGPS),1 stated that “calculated LDL cholesterol concentrations do not change in response to normal food intake in individuals with and without diabetes after correction for hemodilution” (1).

Using unpaired, nonrandomized samples, the authors estimated lipid differences between fasting and nonfasting individuals. Within-individual changes were not measured. Thus, to us, the authors’ conclusions about fasting/nonfasting “changes,” “increases,” or “decreases” were not appropriate. Notably, the maximum difference in the mean triglyceride concentration between fasting and nonfasting individuals with diabetes was 18 mg/dL (0.2 mmol/L). This difference apparently doubled to 35–44 mg/dL (0.4–0.5 mmol/L) (as graphically read) after adjusting for age and sex. We believe that a contrast of this magnitude, apparently arising from covariate adjustments, suggests considerable confounding between fasting and nonfasting individuals. Although Langsted and Nordestgaard did not mention the data in their report, the CGPS had previously reported significant differences between fasting and nonfasting individuals with respect to age, sex, smoking, and so forth (2), underscoring the possible confounding effect of self-selection by participants choosing fasting or nonfasting blood draws. Although the CGPS is a large-scale study, we do not believe that sample size per se affects confounding; it affects only statistical power.

Albumin concentrations in the CGPS were significantly lower in nonfasting individuals than in fasting individuals (approximately 4%–5% lower at 1–2 h postprandially, as graphically read, P < 0.05), as were the concentrations of some lipids, e.g., LDL cholesterol (LDL-C) (approximately 9%–20% lower at 1–2 h postprandially, as graphically read, P < 0.01). After adjustment for albumin, the statistical significance in LDL-C differences disappeared. The CGPS used albumin as a proxy for hemodilution; that is, the CGPS assumed that any difference in the absolute albumin concentrations between individuals was due solely to hemodilution. Like LDL particles, however, albumin is derived from the liver. Mechanisms other than hemodilution (e.g., potentially parallel liver metabolism of albumin and lipoprotein particles) therefore could also explain the association of LDL-C with albumin, a consideration that underscores the limitations of an unpaired, nonrandomized design. Moreover, P values were multiplied, likely by 8 (i.e., 8 independent nonfasting groups), according to the Bonferroni method, with the possibly incorrect aim of adjusting for multiple testing to minimize type I errors (3). Doing so may have minimized the possibility of falsely