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Editorial

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Biological variation: back to basics

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The appreciation of the substantial influence of biological variation (BV) on laboratory testing and interpretation of laboratory results has represented a milestone in the history of laboratory medicine. It has paved the way for a wide series of studies aimed to accurately define the components of BV, set analytical quality specifications and related goals for internal quality control and external quality assessment programs, along with specific criteria for data interpretation, including the reference change value (RCV) [1–3]. The unquestionable importance of quality specifications based on BV in the hierarchy of models defined in the Stockholm Conference in 1999 [4, 5] represented a breakthrough in promoting the generation and application of data on BV. This issue has been for long recognized in *Clinical Chemistry and Laboratory Medicine* as an essential concept for defining the appropriateness of reference values for specific constituents, and for supporting the adoption of RCV in the interpretation of data from serial measurements [6–16]. In this issue of the journal, we publish an article about structure and criteria used for the generation of the BV database [17] along with a commenting Editorial, which are aimed to update our knowledge in this field and encouraging the revision and update of BV database, to reassure that the information is prominently evidence-based [18].

A number of problems are increasingly recognized as a source of debate and concern. First, in an era of global harmonization, mystification should be prevented in the broad range of terms and symbols used to define the components of BV [19]. Therefore, we recognize and support the recent proposition by Simundic et al. to adopt a consistent and harmonized use of terms and symbols as follows [20]:

- CVI: within-subject biological variation (variation within a single individual estimated as a pooled variation from a group of individuals);
- CVG: between-subject biological variation (variation between the central tendencies of a group of individuals);
- CVA: analytical variation (analytical imprecision); should always be clarified, giving mode of derivation and type (such as reproducibility, reliability, or total) and number of analyses, runs, and time period;
- RCV: reference change value (difference required for significance for 2 serial results from an individual); should always be accompanied by the formula used, namely, $2^{1/2}Z\times(CV_A^2\times CV_I^2)^{1/2}$; the *Z*-score should be defined to state the probability and whether unidirectional or bidirectional differences were calculated; and
- II: index of individuality (ratio of analytical and within-subject to between-subject biological variation); should always be accompanied with the formula used for calculation: the preferred $(CV_A^2\times CV_I^2)^{1/2}/CV_G$ or the now seemingly more usual CV_I/CV_G.

Accordingly, the journal will now recommend both authors and referees to comply with this harmonized use of terms and symbols, which are those originally used by Callum G. Fraser and adopted in the papers presented at the Stockholm Conference [3–5]. Further terms and symbols should, therefore, be viewed as simple mistakes or 'deviation from the right way'.

Second, to guarantee the publication of valuable results, clinical investigations on BV should then rely on appropriate study designs, and in particular the reference population inclusion criteria should be carefully specified. Demographic characteristics, (e.g., age, sex and ethnicity), the clinical examination used to establish health in an individual, and subgroups analyses should be declared by authors. Therefore, we also support the suggestion of Aarstand and colleagues to develop an international standard for performing and reporting of studies on BV [18].

As a final consideration, studies should be based on a robust statistical analysis of data. This firstly entails the verification of data distribution, that can be either Gaussian (i.e., normal) or non-Gaussian (i.e., non-normal). In the former instance, parametric analysis can be used [i.e., mean and standard deviation distribution, Pearson's correlation, Student's t-test, and analysis of variance (ANOVA), etc]. As reported by Fraser and Harris [1], nested ANOVA is an efficient way to estimate CV_I. Despite this method not being very sensitive to slightly deviation from normality,

it is highly sensitive to highly aberrant observations. Outliers can be detected by Cochran's test and the Reed's criterion, considering that if an assay is done in duplicate and one measurement is aberrant, the advisable approach is to delete both of them. Moreover, data should be carefully inspected for homoscedasticity before applying ANOVA, as serious violation in homoscedasticity may result in biased estimates and in overestimated goodness of fit. In case of data showing a log-normal rather than a normal distribution, log-transformation should be performed before the analyses as CV_I and CV_G can be calculated by back-transformation. Finally, for data that does meet neither normal nor log-normal distributions, a non-parametric approach could be alternatively used, possibly evaluating the imprecision of estimates (i.e., median and percentile distribution, Spearman's or Kendall's correlation, Mann-Whitney-Wilcoxon test, etc.) [21]. However, as up to now this latter topic has been only marginally studied, we suggest that clinical investigators should concentrate their future efforts to deal with BV calculation on non-normal distributed data.

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