Editorial

Biological variation: a rapidly evolving aspect of laboratory medicine

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Introduction: the need for quantitative data on biological variation

When serial results from examinations of a measurand in laboratory medicine are made in an individual, they are unlikely to be identical over time, even when the state of health or disease in that person has not changed. In part, this is because there are many sources of variation in the pre-examination, examination and post-examination phases of generating a result. However, intrinsic biological variation additionally contributes, often being the most important source of variation. Some measurands have biological variation over the span of life, with important changes at times of rapid physiological development such as the neonatal period, puberty, and the menopause. Others have predictable cyclical variation, which may be daily, monthly, or seasonal in nature. However, most measurands have random variation around homeostatic setting-points (within-subject biological variation), which differ between individuals (between-subject biological variation). Knowledge of the derivation and application of data on both of these components of biological variation are essential for the correct interpretation of results (1,2).

The utility of conventional population-based reference intervals can be determined from the index of individuality, calculated as a ratio of within-subject to between-subject biological variation. Within-subject biological variation and examination imprecision can be used to create reference change values (RCV) to assess the statistical significance of differences in serial results from an individual or to determine the probability that any difference seen is significant. Examination performance specifications for imprecision, bias, total error allowable, measurement uncertainty, and other characteristics can also be created using within-subject and between-subject variation. Thus, generation and subsequent application of numerical data on the components of biological variation are fundamental facets of laboratory medicine.

The generation of numerical data is not without many difficulties and requires expenditure of considerable resources (2,3). In consequence, potential users of data have been much encouraged to use compilations and databases of numerical estimates of within-subject and between-subject biological variation. The creation of a series of comprehensive databases giving one set of values for each measurand for which data were available was initiated in 1997 by the Analytical Quality Commission of the Spanish Society of Clinical Chemistry (SEQC) (4). The last update of this database was in 2014 (5). The database, which has been much cited and widely used, has a number of merits. The suitability of the data prior to inclusion was assessed using an objective scoring system. As at 2014, within-subject biological variation has been documented for 358 measurands in 247 articles. Data are available on measurands in a number of matrices, namely, serum (n=185), plasma (n=74), whole blood (n=55), and urine (n=47). The database was updated every two years, made available on the Internet (5), and includes tabulation of derived examination performance specifications for imprecision, bias, and total allowable error.

Disadvantages of current database and potential improvements

With time, some disadvantages of the widely used database became apparent (6). One problem is the absence of estimates for many measurands of current interest in laboratory medicine. In addition, there has been a relative lack of new publications over recent time for inclusion in the database, with only ca. 25% published since 2000. Moreover, few data are documented for some measurands in that, as at 2014, 202 were found in a single publication, 129 had data in from 2 to 9 publications, and 27 had data in from 10 or more publications. Further, in many publications, duplicate examination results were lacking and assessment of the presence of outliers and the homogeneity of data were not performed. Also, many of the estimates were obtained with what would now be considered obsolete methodology and technology. Additionally, confidence intervals for the estimates, allowing comparison of data, were not generally documented, and thus the robustness of the data is difficult to assess objectively. For a small number of measurands, reviews have been performed on the robustness of published estimates (6), including an impressive analysis of three serum enzyme activities (7), with it being concluded that there was a great variation in the estimates of within-subject variation, probably due to factors including examination methodology, population selection, specimen collection procedures, protocol application, and statistical analyses.

In view of serious professional concerns about the quality of the estimates in the database, an Expert Working Group on Biological Variation of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) produced a checklist to enable standardised production of future publications on biological variation data (8). The checklist identified key elements to be reported in studies to enable safe, accurate, and effective transfer of biological variation data across laboratories. Following this work, a new EFLM Task and Finish Group evolved the checklist, The Biological Variation Data Critical Appraisal Checklist (BIVAC), which can be used to appraise existing studies to be classified according to how well the work fulfils all the required attributes. The results from this currently ongoing appraisal will be used to populate a new database with high-quality estimates and it has been stated that this will be made available on the EFLM website. Moreover, it is hoped that the use of the checklist for new studies would stimulate researchers, authors, reviewers, and journal editors to ensure that studies deliver robust estimates of within-subject and between subject biological variation.

Biological variation of nine serum enzyme activities: a model study

An excellent example of data generated using these up to date approaches has been recently published on nine serum enzyme activities (9). The rationale for the investigation was that examinations of serum enzyme activities are among the most frequently requested in laboratory medicine and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is still much involved in the standardisation of methods for examination of enzyme activities; moreover, Carobene *et al.* (9) considered that examination performance specifications remained to be defined for currently used methodology and technology. Further, another EFLM Task and Finish Group proposed that, of the three models agreed at the 1st EFLM Strategic Conference (10), use of data on biological variation was the appropriate strategy to be used to set examination performance characteristics for these particular measurands (11). Thankfully, the authors used the nomenclature and abbreviations advocated recently, which it is hoped will become universal practice, minimizing confusion (12). In addition, the estimates of within-subject and between-subject biological variation were generated using a number samples collected by six laboratories (Milan, Italy; Bergen, Norway; Madrid, Spain; Padua, Italy; Istanbul, Turkey; Assen, The Netherlands) from 91 apparently healthy subjects, 38 men and 53 women, aged 21–69 years. The samples were collected, importantly ensuring that pre-examination sources of variation were minimized, for a biobank created by the European Biological Variation Study (EuBIVAS) (13). Current methods and guaranteed traceability using reference methods and materials were used to examine enzyme activities, with examination sources of variation minimized, for alanine aminotransferase (ALT) 2.6.1.2, alkaline phosphatase (ALP) 3.1.3.1, α-amylase (AMY) 3.2.1.1, aspartate aminotransferase (AST) 2.6.1.1, creatine kinase (CK) 2.7.3.2, γ-glutamyl transferase (GGT) 2.3.2.2, lactate dehydrogenase (LDH) 1.1.1.27, pancreatic lipase (LIP) 3.1.1.3, and pancreatic α-amylase (PAMY) 3.2.1.1. The data reduction and statistical analyses, a complex issue (14), were comprehensively performed. CV-ANOVA was applied after data were transformed to CV. Then, to assure homogeneity, outlier identification and removal was performed on replicates and samples on the

transformed data. Homogeneity of examination imprecision (between-replicates) was verified using the Bartlett test and homogeneity of within-subject biological variation using the Cochran test. The Shapiro-Wilk test was used to verify the normality of the residuals. For estimation of between-subject biological variation, data were natural log transformed and the Shapiro-Wilk test was again used to verify normality. The Dixon-Reed criterion was used to detect outliers in between-subject means. Further analysis of males and females and country of sample collection were undertaken with statistical rigidity.

It was concluded, quite correctly, that the biological variation data and derived examination performance specifications were generated using current best practice approaches to the pre-examination, examination, and post-examination phases of the work. The results obtained confirmed that the nine serum enzyme activities had high within-subject biological variation and, unsurprisingly perhaps, the estimates were lower than those obtained previously and documented in the 2014 database. In addition, no effects of country were observed, but overall sex-related differences were evident for ALT, GGT, and CK. It was suggested that the derived examination performance specifications could be applied internationally. Overall, it would be very difficult indeed to disagree with the statement of the authors that "*the study design and delivery enabled description of what appears to be the most typical within-subject biological variation with attached confidence limits*".

Overall conclusions

Quantitative data on the components of random biological variation have many uses in laboratory medicine. It is difficult to generate estimates and reliance on the quality of published estimates, particularly in easy to access databases, is an essential prerequisite for good practice. Rational concerns over existing databases (6) have been addressed in a series of recent publications demonstrating model ways to collect samples for studies on biological variation (13), to undertake proper statistical analysis (3,14), to use appropriate nomenclature and abbreviations (12), and to fully document the results (7). It is hoped that researchers, authors, reviewers, and editors will all consider all of these as essential facets of an acceptable study on biological variation. The excellent study of Carobene *et al.* (9) on the biological variation of nine serum enzyme activities should be regarded as the exemplar.

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Footnote

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