Biologic Variation Approach to Daily Laboratory

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KEYWORDS

• Biological variation • Error limits • Quality control • Reference change value

KEY POINTS

- Biologic variation is an unavoidable result of the continuous changes inherent in a living organism and has been studied within subject and between subjects.
- Biologic variation can be used to set the analytical performance specifications for total allowable error, imprecision, and bias (trueness).
- Biologic variation can also be used to assess the significance of changes in serial patient results through a reference change value.
- Biologic variation can be used to determine rules to help autoverification of patient results.
- Recent conferences and studies have made important observations about the validity and usefulness of today's biologic variation estimates. An international effort is working toward improving these estimates.

INTRODUCTION

Laboratory medicine is the science that gives information on the patient health status on the basis of measurements of biological fluids. It is well-known that concentration of analytes in these fluids are not the always exactly at the same concentration owing to the simple fact of being a living, constantly changing organism; this is what in general is named biological variation (BV). Characterization and understanding of BV enables a valid assessment of the significance of a laboratory result.

There are many sources of BV that have been very well-described,[1] with random variation around a central value the focus of this article. There are 2 components of

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random BV: within-subject BV and between-subject BV. Within-subject BV is the random variation around the homeostatic setting point[1] or the random variation that assures an equilibrium state of the human body (data not published[2]). Between-subject BV is the variation among the central points of different individuals. Both terms are usually expressed in terms of percentage coefficient of variation (intraindividual coefficient of variation [CV_I] and intragroup coefficient of variation, respectively).[3]

The way to estimate the components of BV was thoroughly described by Fraser and Harris[4] and data on BV have been compiled in a BV database since 1999 (Ricós-Stockholm conference).[5] This database has been updated every 2 years by the Analytical Committee of the Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC) and has been regularly published at the Westgard website.[6]

More recently, some weaknesses of this database have been described, such as the lack of published studies available for an important number of analytes, discrepancies among the papers that have been compiled, and so on[7,8]; these points were discussed at the European Federation of Clinical Chemistry and Laboratory Medicine Milan Strategic Conference where a Task and Finish group was created with the aim to improve the current database and transform it to a more comprehensive and granulated one as well as reliable as reference data. All the information compiled may be available and will no longer merely the list of quality specifications, but also supporting data such as confidence intervals, and so on.[2,9]

USES OF BIOLOGICAL VARIATION

The currently available data on BV are important information for the medical laboratory that may be used for different applications. These are (1) setting analytical quality specifications, also named performance quality specifications, which are mainly used within the laboratory, (2) assessing the significance of changes in serial results from an individual, reference change value (RCV) that can be used both intralaboratory (delta-check) and can be shown to the clinicians in the laboratory report to inform them about significant changes in patient health status (RCV), and (3) autoverification of results.

Analytical Quality Specifications

Since the Aspen conference in 1977,[10] it has been an accepted standard that the analytical coefficient of variation should be maintained below one-half of the within-subject CV, so that the amount of variability added to the true variability of the result is only about 10%.[11] Further, Gowans stated that when bias is limited below one-quarter of the within-subject plus between-subject coefficients of variation, only a limited percentage of results should be falsely considered outside the upper and lower limits of the population-based reference interval.[12] Accordingly, Ricós and colleagues[13] suggested a limit for total allowable error (TAE) for a single measurement based on the combination of both criteria. The formulae that summarize these statements are:

$$CV_A \leq 0.5 * CV_I$$

$$\text{Bias} \leq 0.25 * (CV_I^2 + CV_G^2)^{1/2}$$

$$\text{TAE} \leq 1.65 * 0.5 * CV_I + 0.25 * (CV_I^2 + CV_G^2)^{1/2}$$

Although the formula for TAE has been debated[14] and some alternatives have been proposed, no revised formula has been generally accepted to date. From the first

Strategic Conference of Milan TFG ("Task Finishing Group") has been created to review and debate this concept.

In addition, Fraser and colleagues[15] suggested also minimum and optimum levels of quality based on fractions different that 0.5 for imprecision and 0.25 for bias, which may be used in the case of testing analytes with very narrow or very wide CV_I according to the current methodological technology.

Nevertheless, the current list of quality specifications from the SEQC group, based on the estimates of CV_I and intragroup coefficient of variation, uses these standard formulae and establishes 3 classes of limits (desirable, minimum, and optimum) so that each laboratory can select the best alternative for its own performance capability. Analytical quality specifications are applied for 2 basic activities of the medical laboratory: internal quality control procedures and evaluation of laboratory performance.

Internal quality control procedure

According to the Westgard's internal quality control protocol, for each analyte and analytical procedure, the laboratory has to estimate its stable performance (in terms of CV_A) and has to define the corresponding quality specification. Briefly, a simple ratio between both terms (6 sigma concept) gives an idea on how restrictive or how relaxed the control rules must be for proper error detection.[16] If any bias exists it should be subtracted from the TAE before calculating the mentioned ratio.[17]

The most important issue is to use the appropriate control rule for each analyte and procedure (even for each control level) instead of using a uniform control rule on all tests in the laboratory (ie, 1:2s). **Table 1** shows an example of a number of analytes tested on a single instrument of a laboratory with different sigma values for the various analytes. The quality specification (desirable) for each analyte is derived from BV; as the TAE narrows, the sigma-metric lowers and more rules and controls are required.

Evaluation of laboratory performance

At a regular interval, the laboratory should review its performance to establish priorities for making improvements and to apply resources when necessary. A simple way to do this is to elaborate monthly figures with CV_A and bias for each analyte and procedure. **Fig. 1** shows an example of serum albumin internal quality control results (at concentration within population based reference values) compared with desirable specifications derived from BV for a 1-year time study.

Problems and solutions applied in daily routine may also be recorded so that requirements for accreditation according to ISO 15189 standard are satisfied.

Laboratory performance is also evaluated and monitoring by participating in external quality assurance (EQA) programs. The recommendations for analytical

Table 1
Control rules for a single instrument used in a stat laboratory

Analyte	Level	Goal	TAE	B_A	CV_A	σ	Rule	N	$P_{de\ (\%)}$	$P_{fr\ (\%)}$
Ferritin	3	BV_{des}	16.9	0.0	1.39	12.2	1:5s	2	90	0
IgG	3	BV_{des}	7.99	0.0	2.37	3.37	1:3s/s:2s.R:4s/3:1s/12x	6	90	8.93
IgA	3	BV_{des}	13.5	0.0	2.39	5.64	1:3.5s	2	90	0.08
IgM	3	BV_{des}	16.8	0.0	2.08	5.46	1:3s	2	90	0.56
Rheumatoid factor	3	BV_{des}	13.5	0.0	2.13	6.32	1:4s	2	90	0.01

Abbreviations: B_A, analytical bias; BV_{des}, desirable biological variation; CV_A, analytical coefficient of variation; P_{de}, probability for detection of error; P_{fr}, probability for false rejection; TAE, total allowable error.

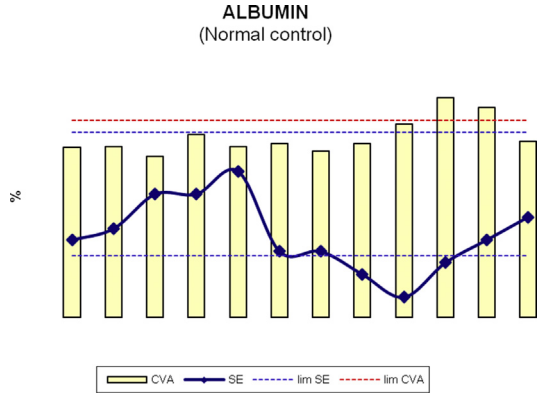

Fig. 1. Monthly CV_A and bias obtained for serum albumin compared with desirable quality specifications. CV_A, analytical coefficient of variation; lim CVA, limit for analytical coefficient of variation; lim SE, limit for systematic error; SE, systematic error.

performance specifications among EQA are not harmonized. Some of them use statistical approaches (United Kingdom National External Quality Assessment Service), others apply state of the art goals aligned with legislated limits of performance (Rilibak, Clinical Laboratory Improvement Amendments), whereas still others recommend biologically derived specifications (Royal College of Pathologists of Australasia).

The most widely used EQA in Spain is organized by the Spanish Society of Clinical Chemistry and Laboratory Medicine (SEQC), which uses acceptability limits for total error derived from BV. Fig. 2 shows a report of serum calcium from a laboratory using

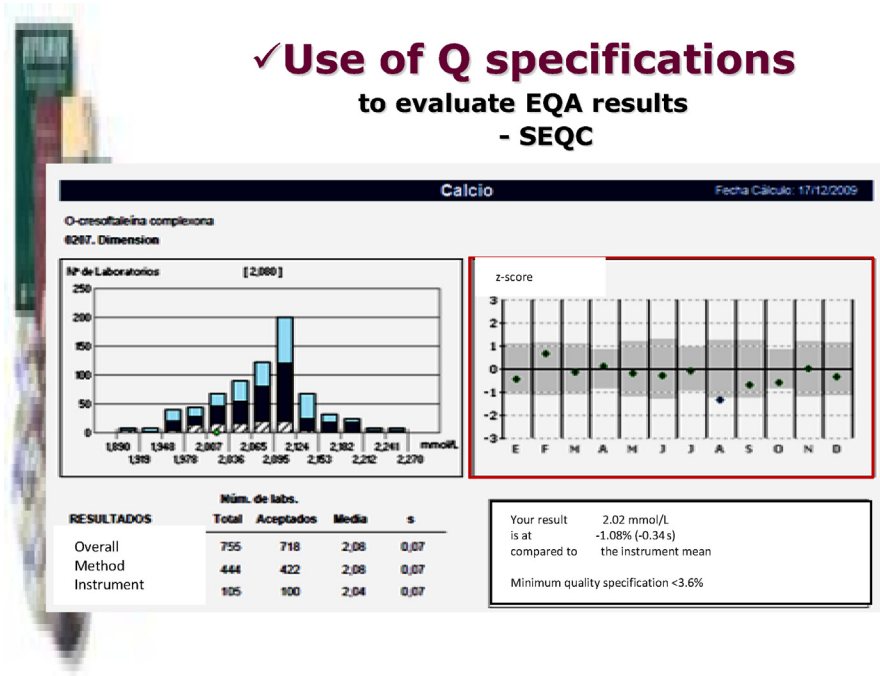

Fig. 2. External quality assurance (EQA) report for serum calcium.

the ortho-cresolftalein method in a Siemens dimension instrument as an example. The left part of the figure shows the frequency histogram of all results and description of total number of results, accepted number of results (after exclusion of outliers), and mean values and standard deviation(s) for overall group and instrument grouped values. The right part shows the deviation of results compared with the instrument mean, expressed both in *z*-score and as a percentage deviation for the last 12 months; also, the minimum specification for total error is shown. Using this report, the laboratory can evaluate its performance (judged as acceptable in the example) for the entire EQA cycle.

Another application of quality specifications is to evaluate performance of all laboratories participating in an EQAS program. **Fig. 3** shows the percentage of results reported to the SEQC-EQA for the basic biochemistry program of 1-year period satisfying the TAE derived from BV (2015).[18] The majority of analytes have 80% or more results within the acceptable limits for total error. The exceptions are acid phosphatase with a high dispersion of results in our setting, sodium and chloride with a very narrow within-subject BV, as well as creatinine with a lot of laboratories using the Jaffé kinetic method, which is nonspecific at levels of creatinine that are less than the upper limit of reference interval, with great impact on the pediatric population.

Reference Change Value

Interpretation of serial results of an analyte in a patient is an important challenge for health care today. Calculation of the individuality index ratio between the within-subject and the between-subject variation gives key information on whether a result from a patient should be compared with the population-based reference interval (the classical procedure) or with previous results from only the same patient and analyte. When the within-subject variability is narrower than the between-subject variability, comparison with previous patient results is much more sensitive to detect changes on health status than comparing with the reference population value.

According to the SEQC database[19] the majority of analytes have high individuality (individuality index <0.6), indicating that a significant change versus the previous result

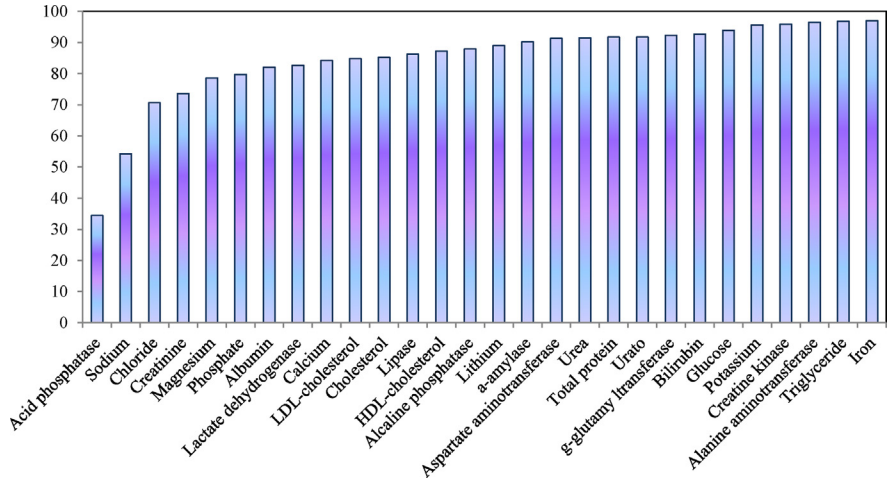

Fig. 3. Percentage of results satisfying the specifications for total allowable error in a year revision. Analytes are shown in ascending order according to the percentage of accomplishment. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

is more important than comparison with the population-based reference value. Laboratories should use this calculation when monitoring patients and incorporate the resulting information into their reports; otherwise, a useful tool for medical decision making could remain hidden.

The formula used to calculate the RCV was proposed by Harris[20]: $RCV = 2^{1/2}Z(CV_A^2 + CV_I^2)^{1/2}$. The Z score determines the level of significance of the change being 1.65 or 2.32 as 95% or 99% of probability for a change for unidirectional changes (1 tailed) and 1.96 or 2.528 for bidirectional changes (2 tailed). Despite 2-tailed Z scores being the most commonly used, the more stringent 1-tailed RCV seems to be more appropriate when a significant increase or decrease is the only change to be considered, as Cooper and colleagues[21] recommended. Fraser published similar advice using an example the assessment of serial observations of cardiac troponins to recognize an acute cardiac event.[22]

When monitoring pathologic status, it is of upmost importance to know the CV_I value in the concrete pathology. That is, we must not only know BV in healthy patients, but the particular variation of analytes in patients with disease. It has been demonstrated that CV_I for a number of analytes, which are the key determinant in some organ-related pathologies, is greater than the CV_I estimated in healthy subjects.[23] This is summarized in **Table 2**.

The use of RCV derived from healthy individuals for monitoring pathologies could lead to an increased risk of false-positive results with the consequent impact on clinical decision making. It is very important to check the distribution of results, because if a skewed distribution is presented, the formula to estimate RCV is different than the formula shown above.

The classical formula to estimate RCV assumes a Gaussian distribution for both CV_A and CV_I; however, it has been observed that a significant number of clinically relevant quantities have a skewed distribution. In these cases, the RCV should be calculated using a lognormal approach that was first described in equations formulated by Fokkema and colleagues.[24] In this approach the total CV_I of non–log-transformed data is used to estimate the σ parameter of the lognormal distribution:

$$\sigma = [\mathrm{Ln}\,(CV_I^2 + 1)]^{1/2}$$

The asymmetrical limits for the upward value for the lognormal RCV (RCV_{pos}) and for the downward value (RCV_{neg}) are determined as follows:

$$RCV_{pos} = [\exp\,(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100$$

$$RCV_{neg} = -[\exp\,(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100$$

In a recent review, Roraas and colleagues[25] recommend the use of the logarithmic method for calculating the RCV regardless of the distribution of data because in statistical terms patient results will always have a certain asymmetry.

Autoverification of Patient Results

Authoverification is a control application within the laboratory aimed at detecting errors that may have been missed by the traditional controls. The formula is: Δ Check $< 2^{1/2} * Z\,(CV_A^2 + CV_I^2)^{1/2}$and Z in this case is recommended to be Z = 1.96 (very significant change). **Fig. 4** shows the diagnostic detection outcome in a primary care laboratory (outpatients). Taking into account that autoverification output is a compromise between sensitivity and specificity to detect errors in requests and results. There is a

Table 2
Analytes with CV_I in pathologies different than in healthy

	Patients				Healthy
Analyte	**Pathology CV_I**	**Type of Pathology**	**Mean**	**Units**	**Subjects CV_I**
α-Amylase	12.4	IDDM	0.50	AE/L	9.5
α-Fetoprotein	38.0	Hepatic diseases	3.97	mg/L	12.0
Alanine aminotransferase	12.6	Chronic liver, IDDM	2.04	μmol/s.L	24.3
Albumin	5.5	Myocardial infarction, IDDM	38.1	g/L	3.1
Albumin concentration. first morning	61.0	Diabetes	14.4	mg/L	36.0
Alkaline phosphatase	12.4	Paget	586	U/L	6.4
CA 125	46.2	Ovarian neoplasia on complete remission	8.77	U/L	29.2
CA 15.3	14.0	Breast cancer patients	16.2	U/mL rev	6.2
CA 19.9	24.5	Ovarian neoplasia on complete remission	8.8	U/L	16.0
CA 19.9	24.5	Lung neoplasia on complete remission	1.3	U/L	16.0
Calcium	4.8	Myocardial infarction	2.25	mmol/L	1.9
CEA	26.9	Breast cancer (operated)	1.6	ng/mL	12.7
CEA	27.0	Breast cancer	ND	ND	12.7
CEA	44.9	Colorectal neoplasia on complete remission	1.99	mg/L	12.7
CEA	23.6	Lung cancer on complete remission	1.77	mg/L	12.7
Creatine kinase	43.3	Inpatients	75.2	U/L	22.8
Creatinine	6.1	IDDM/inpatients	190	μmol/L	4.3
Creatinine	12.3	Renal recipients/ myocardial infarction	85	μmol/L	4.3
C-Telopeptide type I collagen	12.4	Paget	5976	pmol/L	9.6
γ-Glutamyl transferase	4.7	Chronic liver disease	6.8	μmol/s.L	13.4

Abbreviations: CEA, carcinoembryonic antigen; CV_I, intraindividual coefficient of variation; IDDM, insulin-dependent diabetes mellitus; ND, not done.

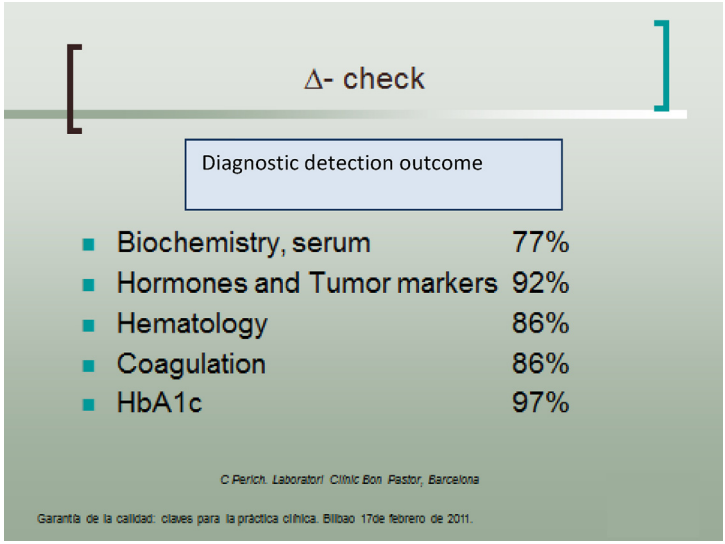

Fig. 4. Delta check diagnostic detection outcome in a primary care laboratory. HbA1c, hemoglobin A1c.

wide dispersion among the output described by different authors: Auxter-Parham[26] found that 70% of detection in oupatients and 10% in hospitalized patients, Dunikoski[27] describes 80% output for biochemistry and coagulation tests, in a hospital laboratory, and Fraser and associates[28] obtained an outcome of 60% in hospitalized patients.

SUMMARY

BV gives valuable information about how the living organism regulates its constituents both within and between subjects; this information on the behavior of body components allows us to derive consequences concerning reference population and reference intervals.

With a more pragmatic approach, BV has a 3 uses: setting the appropriate analytical performance specification for each analyte to limit the amount of error that laboratory could introduce in its measurements, to help to distinguish health from disease (a significant change in a measure and for a particular individual, establishing such a kind of parallelism with current trends of personalized medicine), and, last, to implement internal quality control with the automatic verification of results.

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