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Six Sigma and Calculated Laboratory Tests

To The Editor:

New quality assessment (QA) systems such as Six Sigma have become more popular because they offer a different approach to problems in the clinical laboratory. Laboratory QA programs, however, deal only with measured tests, and thus QA procedures are not applied for most calculated tests, such as measurement of LDL, total globulins, unconjugated bilirubin, creatinine clearance, urea reduction ratio, corrected total calcium, international normalized ratio, plasma osmolality, and anion gap, for which the test results are not measured directly but are calculated based on relationships between measured values *(1)*. We check only the components of calculated tests, not the calculated tests themselves, a practice that may not be adequate for good laboratory management. We should apply QA procedures to all calculated tests and modify the software in clinical laboratories to check the reliability of calculated test results.

The Six Sigma strategy measures the degree to which any process deviates from its goal. Any process can be evaluated in terms of a sigma metric that describes how many sigma fit within the tolerance limits. In quality management, Six Sigma is accepted as "world class quality". For laboratory measurements, the sigma performance of a method can be formulated as below *(2)*:

$$\sigma = \frac{(\text{TEa} - \text{bias})}{\text{CV}} \qquad (1)$$

where TEa is allowable total error.

Because the CVs of calculated tests differ from the CVs of their components *(1)*, we must calculate the CVs of equations. Consider a calculated variable g that is a function of the random variables x, y, and z, i.e., $g = f(x, y, z)$. Now Eq. 1 can be written as follows:

$$\sigma_g = \frac{(\text{TEa}_g - \text{bias}_g)}{\text{CV}_g} \qquad (2)$$

If the variances (SD_i^2) of x, y, and z are known, the variance of g can be approximately obtained by use of the Taylor series expansion of the function *(3)*. If $g = f(x, y, z)$ is approximately linear with respect to x, y, and z in the region of interest, the approximate variance of g is obtained as:

$$\text{SD}_g^2 \cong (\delta_g/\delta_x)^2\text{SD}_x^2 + (\delta_g/\delta_y)^2\text{SD}_y^2 + (\delta_g/\delta_z)^2\text{SD}_z^2 \qquad (3)$$

where x, y, and z are the individual means of each test.

If we divide both side of this equation by g^2 and cancel the common terms, we obtain the following equation:

$$\text{CV}_g^2 = \text{CV}_x^2 + \text{CV}_y^2 + \text{CV}_z^2 \qquad (4)$$

This equation is valid only if the variables (measured components of equation) are independent.

Eq. 4 can be used to obtain the CVs of calculated tests. Alternatively, we may observe CV empirically from the results of measured control materials on control charts.

Combining Eqs. 2 and 4, we get a new equation:

$$\sigma_g = \frac{(\text{TEa}_g - \text{bias}_g)}{(\text{CV}_x^2 + \text{CV}_y^2 + \text{CV}_z^2)^{1/2}} \qquad (5)$$

Under ideal conditions for a reference method, bias can be assumed to be zero if the method is properly calibrated. Otherwise, we must calculate total bias (bias of equation). In this situation, Eq. 5 can be further simplified:

$$\sigma_g = \frac{\text{TEa}_g}{(\text{CV}_x^2 + \text{CV}_y^2 + \text{CV}_z^2)^{1/2}} \qquad (6)$$

From Eq. 4, it is obvious that the CV of the equation is higher than the CV of any component of the equation. Thus, if $\text{TEa}_g \leq \text{TEa}_i$, σ_g will be lower than σ_i (where i represents the measured tests that are components of the equation, such as x, y, and z).

We can examine σ_g for LDL as an example.

Serum LDL concentrations can be obtained indirectly by use of the Friedewald equation *(4)*:

$$\text{LDL-cholesterol} = \text{TC} - [\text{HDL} + (\text{triglycerides}/5)] \qquad (7)$$

where TC is total cholesterol. The factor (triglycerides)/5 is an estimate of VLDL-cholesterol concentration and cannot be used unless the serum triglyceride concentration is <4.48 mmol/L (4000 mg/L).

To apply Eq. 4, we obtain the approximate CV of LDL:

$$(\text{CV}_{\text{LDL}})^2 = (\text{CV}_{\text{TC}})^2 + (\text{CV}_{\text{HDL}})^2 + (\text{CV}_{\text{VLDL}})^2 \qquad (8)$$

With a given TEa, which is recommended by CLIA or the College of American Pathologists (CAP), the sigma of calculated LDL will be lower than the sigma of measured LDL. As shown in Table 1, the CV of calculated LDL (7.4%; obtained with Eq. 8) is higher than the CV of mea-

Table 1. TEa (recommended by CAP) and desirable CV to obtain 6 σ for measured lipids (bias accepted as zero).

Test	TEa (recommended by CAP), %	Desirable CV to obtain 6 σ, %
Total cholesterol	10	1.7
HDL	30	5.0
Triglycerides	25	4.2
LDL	20	3.3

sured LDL (3.3%). Thus the CV of measured components should be lower to obtain an acceptable CV for calculated LDL and thus reach the Six Sigma goal. Otherwise, we must measure the serum LDL concentration by chemical methods.

In conclusion, when a QA process is implemented in the clinical laboratory, application of that process only to measured tests is inadequate. Because of their higher CVs, results of calculated tests have lower precision than those of measured tests. Thus, Six Sigma world-class quality may be difficult to attain for calculated tests.

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Validation of Breast Cancer Biomarkers Identified by Mass Spectrometry

To the Editor:

Li et al. *(1)* should be congratulated for a valiant effort to validate 3 previously identified serum breast cancer biomarkers by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS).

Because there is considerable controversy on the value of this technology for cancer diagnostics *(2–11)*, it is important to comment on validation studies aiming to reproduce previously published data. Among 3 previously reported biomarkers, BC1, BC2, and BC3, one of these (BC1) was not confirmed, as it was previously shown to be decreased in breast cancer, whereas in the validation study by Li et al. *(1)*, it was increased.

The other 2 candidate biomarkers, BC2 and BC3, were positively identified, by tandem MS, as complement C3a lacking its C-terminal arginine ($C3a_{desArg}$). BC2 was also identified as a truncated form of $C3a_{desArg}$.

In my opinion, the data presented in Fig. 4 of the article by Li et al. *(1)*, showing the relative intensities of BC2 and BC3 in various groups of patients, are rather disappointing. For BC2, there is no difference between patients with benign breast diseases and patients with invasive carcinomas, although an increase was seen in ductal carcinoma in situ (DCIS). For BC3, there was no difference among patients with benign disease, DCIS, or invasive carcinomas.

The remaining question concerns the possible value of complement $C3a_{desArg}$ and its fragment as candidate breast cancer biomarkers. The data provided by the authors *(1)* confirm my previous predictions that SELDI-TOF–identified biomarkers represent high-abundance proteins (in this case, C3, present in serum at concentrations of ~1.2 g/L) that are produced mostly by the liver *(3–6)*. The proteolytic processing of peptides in the circulation by amino- and carboxypeptidases is well known, and it should not be surprising that the identified molecules represent modified and/or truncated forms of C3a.

I have previously speculated that a large number of SELDI-TOF–identified candidate biomarkers are acute-phase reactants *(3–6)*. C3, in accordance with my previous predictions, is also an acute-phase reactant whose serum concentration is increased or decreased in a wide variety of clinical conditions *(12)*.

I conclude that the positive identification of previously described candidate serum biomarkers, BC2 and BC3, confirms my previous predictions that these are high-abundance proteins produced by the liver and that they represent nonspecific biomarkers of acute-phase reaction. Their performance as breast cancer biomarkers, as assessed by SELDI immunoassay, is not impressive and likely of questionable clinical value.

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