INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2024.75768
Int J Med Biochem 2024;7(3):136–142

Research Article

Evaluation of the analytical performance of 80 parameters analyzed in routine biochemistry laboratory by process sigma methodology

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Abstract

Objectives: The Six Sigma methodology is also frequently used by clinical laboratories as an objective and quantitative way to measure quality. In our study, we aimed to evaluate the analytical performance of 80 tests using the Six Sigma methodology according to the CLIA (Clinical Laboratory Improvement Amendments) 2019, RICOS BV (Dr. Carmen RICOS Biological Variation) Desirable, and EFLM BV (European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation) Desirable criteria.

Methods: The sigma values of 80 tests were calculated according to the TEa (Total Allowable Error) limits allowed by all three references using internal quality control and external quality control data. They were calculated monthly for 12 months, and the annual average was taken. Sigma values were calculated with the Six Sigma formula.

Results: Considering the total number of goals reached, the highest success rate of 60% was achieved according to the CLIA goals, while the lowest success rate of 36% was obtained according to the EFLM BV Desirable criteria. Although exactly the same laboratory data are used, this gap between the sigma values obtained according to the selected reference is especially noticeable in tests such as Na (Sodium), K (Potassium), Cl (Chloride), Calcium, HbA1c (Hemoglobin A1c), and Troponin T.

Conclusion: The Six Sigma protocol is one of the effective and universal tools for evaluating the performance of clinical laboratories. However, one of its biggest limitations is the lack of standardization in tolerance limits. The obtained performance varies according to the preferred reference. Therefore, we think that in the Six Sigma methodology, it is more feasible to select Total Allowable Error criteria from different references according to their suitability for the test.

Keywords: Biological variation, CLIA 2019, quality control, RICOS BV, six sigma, total allowable error

How to cite this article: Esen M, Karahan SC, Orem A, Aliyazicioglu Y, Bozkurt Yavuz H. Evaluation of the analytical performance of 80 parameters analyzed in routine biochemistry laboratory by process sigma methodology. Int J Med Biochem 2024;7(3):136–142.

Laboratory tests are performed on approximately 85% of individuals who present to healthcare facilities [1]. Since medical laboratory service, which is an important part of health services, is directly related to patient health, the accuracy of the results obtained from the medical laboratory and the correct functioning of the processes are crucial. The mortality rate due to laboratory errors varies from 0.05% to 0.61% [2]. Laboratory errors can lead to delayed diagnoses, misdiagnosis, incorrect treatment, increased risk to patient safety, increased costs, and lost time [3]. Medical laboratory processes are generally divided into three phases: preanalytical, analytical, and postanalytical. When evaluating laboratory errors by their phase, it has been shown that most errors occur in the preanalytical or postanalytical phase and few occur in the analytical phase [4]. Nevertheless, analytical quality is of critical importance to laboratories.

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Submitted: December 27, 2023 **Revised:** May 27, 2024 **Accepted:** May 29, 2024 **Available Online:** August 15, 2024

Analytical error is the difference between the observed value and the true value and is divided into random error and systematic error. The random error can be negative or positive, its direction and magnitude are unpredictable, which is indicated by imprecision (CV) [5]. The systematic error, on the other hand, is an error with definite and measurable values that change the analysis result at a fixed and definite level. It always occurs in one direction [5]. It affects the accuracy of the analysis result and is indicated by bias. The sum of the random error and the systematic error is expressed as the total error (TE) and calculated with the formula 'Total Error = Bias+1.65xCV%' (equation-1) [5]. The total allowable error (TEa) for each test and its magnitude have been established as performance criteria by some organizations [6–8].

The Six Sigma methodology is used as an objective and quantitative method for measuring quality. Sigma expresses the frequency of defects as "defects per million possibilities (DPM)." A 6-sigma performance corresponds to 3.4 errors per million measurements, which is considered a world-class performance. Bias, CV, and TEa can be used to evaluate the quality of the analytical phase (Sigma value = (%TEa-%Bias) / CV%) (equation-2) [5]. The minimum acceptable performance for medical laboratories is a 3-sigma performance [5]. Different TEa limits are being used [6–8]. In this study, we aimed to evaluate the analytical performance of 80 tests studied in a clinical biochemistry laboratory by the Six Sigma method calculated with different TEa limits. Although there are many studies in the literature that apply the Six Sigma methodology to clinical chemistry testing in particular, it is very difficult to come across a study that holistically addresses such a broad test profile, almost all of the laboratory's tests, using up-to-date TEa resources.

Materials and Methods

This study was conducted in the Clinical Biochemistry Laboratory of the Faculty of Medicine, Karadeniz Technical University, Farabi Hospital, with the approval of the Ethics Committee number 2014-168. Internal quality control and external quality control data for the period January 2014 to December 2014 were used in our study. Clinical chemistry parameters, hemogram, Hemoglobin A1c (HbA1c), immunoassay parameters, specific proteins, prenatal screening tests, coagulation parameters, and cardiac markers were included in the study. Thus, the annual average six sigma values of 80 parameters analyzed in the mentioned laboratory were calculated separately for each internal quality control level.

Analysis of routine clinical chemistry parameters was performed with a Beckman Coulter AU 5800 autoanalyzer, and that of the HbA1c test with a Biorad D10 autoanalyzer. The analysis of hemogram parameters was performed with a Beckman Coulter LH 780 autoanalyzer, and the analysis of prenatal screening parameters and the tests of DHEA-S (Dehydroepiandrosterone sulfate), TG (Thyroglobulin), ATG (Anti-thyroglobulin), ATA (Anti-thyroperoxidase), C-peptide, IGF-1 (Insulin-like growth factor 1), insulin, and PTH (Parathyroid hormone) were performed with a Siemens Immulite2000XPi autoanalyzer. Analysis of other immunoassay tests was performed with the Beckman Coulter DXI 800, other specific protein parameters were analyzed with the Siemens BN-II, coagulation parameters were analyzed with the Stago STAR Evaluation, and cardiac parameters were analyzed with the Roche Cobas Integra e 411.

In order to evaluate Albumin, ALP (Alkaline phosphatase), ALT (Alanine aminotransferase), amylase (AMY), AST (Aspartate aminotransferase), d.bil (Direct (Conjugated) bilirubin), t.bil (Total bilirubin), t.Ca (Total calcium), Cl (Chloride), HDL-K (High-density lipoprotein cholesterol), LDL-C (Low-density lipoprotein cholesterol), t.cholesterol (Total cholesterol), CK (Creatine kinase), creatinine, GGT (γ-glutamyltransferase), glucose, iron (Fe), LDH (Lactate dehydrogenase), Mg (Magnesium), PO4 (Phosphate), K (Potassium), t.protein (Total protein), Na (Sodium), TG (Triglycerides), urea, uric acid (u.acid) parameters (lot numbers: 51872, 51873) and Hemogram tests (lot numbers: 878000, 866400, 887900) Beckman Coulter internal quality control materials were used as internal quality control (IQC) material. IQC of the HbA1c parameter was performed with BIO-RAD Lyphocheck R Diabetes Control solution (lot numbers: 33871, 33872). IQC of cortisol, estradiol, ferritin, folate, FSH (Follicle-stimulating hormone), LH (Luteinizing hormone), HCG (Human chorionic gonadotropin), prolactin, total PSA (Total prostate-specific antigen), free T3, free T4, testosterone, TSH (Thyroid stimulating hormone), IGE (Immunoglobulin E), DHEA-S parameters were performed with BIO-RAD Lyphocheck R Immunoassay Plus Control (lot numbers: 40271, 40272).

IQC of AFP (α-feto protein), CA 125 (Cancer antigen 125), CA 15–3 (Cancer antigen 15–3), CA 19–9 (Cancer antigen 19–9), CEA (Carcinoembryonic antigen), and total PSA were performed using the BIO-RAD Lyphocheck R Tumor Marker Plus control (lot numbers: 54571, 54572). IQC of TG, ATG, ATA, C-peptide, IGF-1, insulin, PTH (lot numbers: 0212014111, 0212014112, 0212014113). CRP (C-Reactive protein), C3 (Complement 3), C4 (Complement 4), IGA (Immunoglobin A), IGG (Immunoglobin G), IGM (Immunoglobin M) were performed with Siemens IQC samples (lot numbers: 084742, 084744, 084745). Randox Maternal Control Material was used for IQC of maternal screening parameters AFP, HCG total, and PAPP-A (Pregnancy associated plasma protein A) (lot numbers: 1272015021, 1272015022). IQC of fibrinogen, aPTT (Activated partial thromboplastin time), PT (Prothrombin time) were performed with IQC samples from Stago (lot numbers: 108886, 111551). IQC of CK-MB (Creatine kinase muscle brain isoenzyme), myoglobin, NT -ProBNP (N terminale Pro-brain natriuretic peptide), Troponin T was performed with IQC samples from Roche (lot numbers: 173608, 173609).

External quality assurance (EQA) of biochemical tests, hemogram, HbA1c, specific proteins and immunoassays (except DHEA-S, TG, ATG, ATA, C-peptide, IGF-1, insulin, PTH) are performed with BIO-RAD EQAS (External Quality Assurance Services (USA)). EQA for immunoassay parameters, specific pro-

Table 1. One-year average of CV% values of internal quality controls and bias% values of external quality control and process sigma values of Biochemistry and HbA1c tests

Test	Bias%	CV%		Process sigma CLIA		Process sigma RICOS (desirable)		Process sigma EFLM BV (Desirable)	
		L1	L2	L1	L2	L1	L2	L1	L2
Albumin (g/L)	2.1	2.4	2.1	2.5	2.8	0.8	0.9	0.5	0.6
ALP (U/L)	1.6	5.1	4.4	3.6	4.2	2.0	2.4	1.7	2.0
ALT (U/L)	1.6	3.0	2.2	4.5	6.1	8.6	12.0	4.8	6.6
AMY (U/L)	1.7	2.1	1.9	4.0	4.4	6.1	6.7	5.5	6.1
AST (U/L)	2.3	2.6	2.1	4.9	6.0	5.5	6.8	4.3	5.4
D.Bil (mg/dL)	2.4	2.3	2.5	–	–	18.1	16.6	9.7	9.1
T. Bil (mg/dL)	2.9	2.9	2.4	5.9	7.1	6.3	7.1	7.6	9.1
T. Calcium (mg/dL)	1.5	1.8	2.1	5.7	5.1	0.6	0.5	0.4	0.4
Cl (mEq/L)	0.7	1.3	1.4	3.3	3.2	0.6	0.6	0.5	0.4
HDL-C (mg/dL)	2.4	3.7	3.0	4.8	5.9	2.5	3.1	2.4	2.9
LDL-C (mg/dL)	5.9	2.7	2.8	5.2	5	2.2	2.2	2.9	2.8
T. Cholesterol (mg/dL)	3.5	2.4	2.3	2.7	2.8	2.3	2.4	2.2	2.3
CK (U/L)	1.7	2.5	2.2	7.3	8.3	11.4	13.1	8.4	9.5
Creatinine (mg/dL)	4.9	3.2	2.6	1.6	2.0	1.2	1.5	0.8	1.1
GGT (U/L)	1.3	2.1	2.3	6.5	6	8.9	8.9	8.4	7.7
Glucose (mg/dL)	2.2	1.6	2.0	3.6	2.9	2.3	2.4	2.7	2.2
Iron (µg/dL)	3.6	1.6	1.9	7.1	6.0	10.4	14.2	14.4	12.2
LDH (U/L)	1.9	1.7	2.9	7.7	4.5	2.3	3.3	3.4	2.1
Mg (mg/dL)	2.2	2.3	2.2	5.6	5.8	1.0	1.2	0.8	0.8
PO4 (mg/dL)	2.3	2.4	3.4	3.2	2.3	2.5	2.3	3.1	2.2
K (mEq/L)	0.8	2.9	1.3	2.4	1.7	3.9	3.6	1.4	3.1
T. protein (g/L)	1.6	1.5	2.2	4.3	2.9	0.9	0.9	1.3	0.9
Na (mEq/L)	0.6	0.7	1.0	2.4	2.6	0.2	0.2	0.1	0.1
TG (mg/dL)	1.9	2.4	3.0	5.5	4.4	8.3	8.1	10.5	8.4
Urea (mg/dL)	2.2	3.3	2.9	2.1	2.3	4.1	4.6	4.7	5.4
Uric acid (mg/dL)	2.2	3.5	2.0	2.2	3.9	4.3	4.9	–	–
HbA1c (%)	2.4	4.9	1.2	1.6	6.3	0.3	0.5	Negative	Negative

CV: Coefficient of variation; CLIA: Clinical Laboratory Improvement Amendments; EFLM BV: European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase: AMY: Amylase; AST: Aspartate aminotransferase; D. Bil: Direct (Conjugated) bilirubin; T.Bil: Total bilirubin; T. Calsium: Total calcium; Cl: Chloride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; T. Cholesterol: Total cholesterol; CK: Creatine kinase; GGT: γ- glutamyltransferase; LDH: Lactate dehydrogenase; Mg: Magnesium; PO4: Phosphate; K: Potassium; T.protein: Total protein; Na: Sodium; TG: Triglycerides; HbA1c: Glycated hemoglobin.

teins, prenatal screening tests, coagulation parameters and cardiac markers were performed with RIQAS (Randox International Quality Assessment Scheme (UK)) Control.

The one-year process sigma values of the relevant tests were calculated monthly, separately for each control level, and the annual average was taken. The process sigma was calculated according to equation-2. For the CV % value, the IQC data of each test were used and the arithmetic annual average was taken. Peer group bias % values obtained from the external quality control reports of each test were recorded regularly for each month and the annual arithmetic mean was calculated. The monthly EQA reports of the tests were accessed through the websites of the relevant EQA programs (www.qcnet.com and www.riqas.net) by the user code and password of the laboratory. Process sigma values were calculated separately based on the TEa according to CLIA (Clinical Laboratory Improvement Amendments) 2019, Ricos BV (Dr. Carmen Ricos Biological Variation) Desirable, and EFLM BV (European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation) Desirable [6–8].

Results

The 1-year target value averages and the CV% values of IQC of all tests included in the study, the bias% values obtained from the external quality control results, and the average of process sigma values for the internal quality control levels calculated according to CLIA 2019, RICOS BV desirable, and EFLM BV desirable criteria are shown in Tables 1-3.

Table 2. One-year average of mean, standard deviation and CV% values of internal quality controls and bias % values of external quality control and process sigma values of hemogram, coagulation, cardiac marker, prenatal screening, specific protein tests

Test	Bias%	CV%			Process sigma CLIA			Process sigma RICOS (Desirable)			Process sigma EFLM BV (Desirable)		
		L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
HCT (%)	1.8	0.6	0.7	0.8	3.7	1.1	2.8	3.4	3.0	2.8	3.5	3.0	2.6
HGB (g/dL)	0.7	0.5	0.5	1.0	6.6	6.6	3.3	7.2	6.5	3.4	6.2	6.2	3.1
MCH (pg/cell)	2.0	0.6	0.8	1.0	–	–	–	0.8	0.6	0.5	<0	<0	<0
MCHC (g/dL)	1.6	0.7	0.8	1.1	–	–	–	<0	<0	<0	<0	<0	<0
MCV (fL)	2.8	0.5	0.6	0.5	–	–	–	<0	<0	<0	<0	<0	<0
MPV (fL)	2.6	0.7	0.7	0.7	–	–	–	4.6	4.8	4.9	1.7	1.7	1.7
PLT (K/µL)	4.4	1.5	1.6	2.3	13.7	12.9	9.0	5.9	5.8	4.0	4.6	4.3	3.0
RBC (M/µL)	1.2	0.5	0.5	0.6	5.6	5.6	4.7	6.9	6.6	5.2	5.4	5.4	4.5
RDW (%)	1.7	1.2	1.3	1.4	–	–	–	2.4	2.1	2.0	0.8	0.7	0.6
WBC (K/µL)	2.5	1.2	1.4	2.3	2.1	1.8	1.1	10.6	9.4	5.7	9.4	8.1	4.9
CK-MB (µg/L)	3.7	4.5	4.7	–	4.7	4.5	–	5.9	5.6	–	–	–	–
Myog (ng/mL)	3.5	3.7	3.6	–	–	–	–	4.4	4.5	–	–	–	–
NT-ProBNP (pg/mL)	3.0	3.3	3.2	–	8.2	8.4	–	3.0	3.1	–	–	–	–
Troponin T (ng/mL)	9.4	4.6	2.5	–	4.5	8.2	–	8.6	15.8	–	1.8	3.3	–
Fibrinogen (mg/dL)	3.0	5.7	6.7	–	3.0	2.5	–	1.9	1.6	–	–	–	–
a PTT (sn)	6.0	3.2	3.6	–	2.8	2.5	–	<0	<0	–	–	–	–
PT (sn)	3.9	4.5	5.3	–	2.5	2.1	–	0.3	0.3	–	–	–	–
AFP (IU/mL)	4.9	4.7	4.8	–	3.2	3.1	–	3.6	3.6	–	2.7	2.6	–
Total HCG	7.2	4.1	4.9	–	2.6	2.2	–	–	–	–	–	–	–
PAPP-A (mIU/mL)	4.6	5.5	5.1	–	–	–	–	1.9	2.1	–	–	–	–
C3 (g/L)	5.5	5.0	4.5	5.0	1.9	2.1	1.9	0.6	0.6	0.6	0.5	0.5	0.5
C4 (g/L)	3.0	5.2	5.2	5.1	3.3	3.3	3.3	2.5	2.5	2.5	1.8	1.8	1.8
IGA (g/L)	5.4	4.8	4.3	5.0	2.0	2.2	1.9	1.7	1.9	1.6	0.9	1.0	0.9
IGG (g/L)	6.2	4.7	4.7	4.6	2.9	2.9	3.0	0.4	0.4	0.4	0.2	0.2	0.2
IGM (g/L)	6.1	4.3	5.4	5.1	3.2	2.6	2.7	2.5	2.0	2.1	2.6	2.0	2.2
IgE (IU/mL)	3.0	5.3	5.5	–	3.2	3.1	–	–	–	–	–	–	–

CV: Coefficient of variation; CLIA: Clinical Laboratory Improvement Amendments; EFLM BV: European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation; HCT: Hematocrit; HGB: Hemoglobin; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MPV: Mean platelet volume; PLT: Platelet; RBC: Red blood cells; RDW: Red cell distrubition width; WBC: White blood cells; CK-MB: Creatine kinase muscle brain isoenzyme; Myog: Myoglobin; a PTT: Activated partial thromboplastin time; PT: Prothrombin time; AFP: α-feto protein; Total HCG: Total human chorionic gonadotropin; PAPP-A: Pregnancy associated plasma protein A; C3: Complement 3; C4: Complement 4; IGA: Immunoglobin A; IGG: Immunoglobin G; IGM: Immunoglobin M; IgE: Immunoglobulin E.

Discussion

For many years, studies have been carried out and applied to evaluate and improve the quality of the analytical process. The Six Sigma protocol is one of the most effective and universal tools for evaluating clinical laboratory performance. The sigma value of a test is a numerically defined value for the quality measure of that test [9, 10].

For all tests in our study, unacceptable performance (<3 sigma) was observed in 54 of the 130 targets according to the CLIA criteria, 89 of the 172 targets according to the RICOS BV Desirable criteria, and 86 of the 133 targets according to the EFLM BV Desirable criteria. In this case, the success rate of the laboratory is 60% according to CLIA, 49% according to RICOS BV Desirable criteria, and 36% according to EFLM BV Desirable criteria. In the literature, there are studies that have similar results to our study, and it is possible to come across studies that have higher or lower performance than ours [11–19].

Process sigma analyses can be used effectively to evaluate analytical performance and govern internal quality control procedures, but some limiting factors should be considered when calculating the sigma value. The first of these factors is the method used to calculate the bias. As is well known, the best method for calculating bias is to use a reference material/method [12]. However, for many analytes, there is no reference method or reference material, and even if there is, it is very difficult for clinical laboratories to obtain. Therefore, it is very difficult to calculate the bias realistically. In practice, the percentage bias can be calculated using internal quality control data or external quality control data [9, 11].

Table 3. One-year average of mean, standard deviation and CV% values of internal quality controls and bias % values of external quality control and process sigma values of hormon and tumor markers

Test	Bias%	CV%			Process sigma CLIA			Process sigma RICOS (Desirable)			Process sigma EFLM BV (Desirable)		
		L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
AFP (ng/mL)	5.7	5.3	5.6	–	2.7	2.6	–	3.1	2.9	–	2.2	2.1	–
CA 125 (U/mL)	4.5	4.4	4.7	–	3.5	3.3	–	7.0	6.5	–	2.1	2.0	–
CA 15–3 (U/mL)	3.7	4.6	4.6	–	–	–	–	3.7	3.7	–	–	–	–
CA 19–9 (U/mL)	4.2	4.5	4.8	–	–	–	–	9.2	8.8	–	3.0	2.9	–
CEA (ng/mL)	4.1	5.1	4.9	–	–	–	–	4.0	4.2	–	3.2	3.3	–
Cortisol (µg/dL)	2.9	7.1	5.3	–	2.4	3.2	–	2.8	3.8	–	3.3	4.4	–
Estradiol (pg/mL)	5.7	12.6	9.1	–	1.9	2.7	–	1.7	2.3	–	0.9	1.3	–
Ferritin (ng/mL)	9.6	5.6	5.2	–	1.9	2.0	–	1.3	1.4	–	–	–	–
Folate (ng/mL)	2.6	4.4	4.0	–	6.2	6.9	–	8.2	9.2	–	–	–	–
FSH (mIU/mL)	3.8	4.8	5.0	–	3.0	2.8	–	3.6	3.5	–	3.6	3.5	–
HCG (mIU/mL)	4.0	5.3	5.1	–	2.6	2.7	–	–	–	–	–	–	–
LH (mIU/mL)	4.2	6.7	6.3	–	2.4	2.5	–	3.6	3.8	–	3.6	3.8	–
Prolactin (ng/mL)	3.4	3.6	4.2	–	4.6	4.0	–	7.2	6.2	–	9.4	8.1	–
Total PSA (ng/mL)	3.7	8.2	4.5	–	2.0	3.6	–	3.6	6.7	–	1.5	2.8	–
Free T3 (pg/mL)	3.7	6.4	6.4	–	–	–	–	1.2	1.2	–	0.4	0.4	–
Free T4 (ng/dL)	4.3	5.8	4.6	–	1.8	2.3	–	0.6	0.8	–	0.3	0.4	–
Testosterone (ng/mL)	4.5	3.9	3.9	–	4.0	4.0	–	2.3	2.3	–	3.1	3.1	–
TSH (µIU/mL)	4.6	5.4	5.4	–	2.9	2.9	–	3.5	3.5	–	3.7	3.7	–
CRP (mg/dL)	4.1	4.7	4.5	–	5.5	5.8	–	11.9	12.5	–	9.9	10.4	–
DHEA-S (µg/dL)	2.0	5.8	5.5	4.8	–	–	–	1.9	2.0	2.3	1.4	1.5	1.8
TG (ng/mL)	9.9	8.3	5.3	5.3	–	–	–	1.4	2.3	2.3	2.2	3.5	3.5
ATG (U/mL)	11.6	8.1	7.7	–	–	–	–	2.0	2.1	–	–	–	–
ATA (U/mL)	8.6	8.9	6.6	–	–	–	–	4.2	5.7	–	–	–	–
C peptide (ng/mL)	8.7	4.3	3.8	4.3	–	–	–	2.8	3.2	2.8	–	–	–
IGF-1 (ng/mL)	11.6	5.5	4.9	–	–	–	–	2.3	2.5	–	0.6	0.7	–
İnsulin (mU/L)	22.1	4.0	3.3	–	–	–	–	2.7	3.2	–	2.4	2.8	–
PTH (pg/mL)	9.3	4.2	5.1	–	–	–	–	5.0	4.0	–	2.5	2.1	–

CV: Coefficient of variation; CLIA: Clinical Laboratory Improvement Amendments; EFLM BV: European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation; AFP: α-feto protein; CA 125: Cancer antigen 125; CA 15-3: Cancer antigen 15-3; C A19-9: Cancer antigen 19-9; CEA: Carcinoembryonic antigen; FSH; Follicle- stimulating hormone; HCG: Human chorionic gonadotropin; LH: Luteinizing hormone; Total PSA: Total prostate spesific antigen; TSH: Thyroid stimulating hormone; CRP: C-Reactive protein; DHEA-S: Dehydroepiandrosterone sulfate; TG: Thyroglobulin; ATG: Anti-thyroglobulin; ATA: Anti- thyroperoxidase; IGF-1: Insulin-like growth factor 1; PTH: Parathyroid hormone.

While calculating the bias% value we used for each test in this study, we took the group mean value in the external quality control data as a basis. In similar studies in the literature, the bias value used for sigma calculation was obtained from the mean values of the external quality control group [9, 12, 15, 17, 19]. The point to be noted here is that the group mean values in the external quality control data are the mean values of the data, including all group participants. In other words, it is not a value determined by analyzing the reference method. Therefore, the bias value we calculated is not actually 'true bias', but maybe 'relative bias'. In addition, there are also studies using internal quality control data for bias calculation [11, 14, 16]. Some researchers argue that using IQC data will be more accurate than using EQA data, because the EQA group mean value is affected by the measurement uncertainty of all group participants, and they recommend using IQC data in the calculation of bias unless a comparison is made with the reference method [11]. We believe that using IQC bias will also be a biased approach and will not be sufficient to show objective and true bias. However, as part of routine laboratory operations, the use of reference material to calculate sigma values for all tests is not a cost-effective approach, so we preferred the use of EQA bias values. We believe that the use of bias from IQC is a barrier to comparing our results with those of other laboratories.

Another limiting factor is that the CV% values are related to the concentrations. In most cases, the CV% of a parameter is high at low concentrations and decreases as the concentration increases. Therefore, it is possible that different performances are obtained at different concentrations [20]. This

difference was also observed in our study. For example, as the CV% of the total PSA assay is high at low concentrations, the sigma value varies between 1.5 and 3.6, whereas its performance is at a world-class level at high concentrations. This is true for almost all parameters. In this case, the question of which CV % value to use for calculating performance can be answered by considering clinical decision limits.

One of the problems that cause limitations in calculating the sigma values of the tests is the use of control materials instead of patient samples. There is a matrix difference between control materials and real patient samples, so the analytical responses of control materials to the measurement of a test may not be uniformly matched to the patient sample [14]. On the other hand, stability can also be an issue in CV studies conducted with patient serum pools. Therefore, we recommend using internal quality control samples.

Another point to consider is that when calculating the analytical performance of a test, different sigma values are obtained according to the selected TEa reference (such as CLIA, RICOS BV, EFLM BV). Although the same Bias% and CV% values were used in our study, it was observed that different sigma values would be obtained according to the selected reference. For example, while the process sigma value of the Troponin T test was calculated with CLIA and RICOS, it had higher sigma values (between 4.5 and 15.8); when calculated with EFLM BV, the low concentration level showed unacceptable performance, and the sigma value of the high concentration level was on the borderline.

The TEa criteria of Biological Variation are often more stringent and their relevance is controversial. Tests such as electrolytes, HbA1c, and coagulation tests can be placed in this group. For example, the desirable limit for Sodium is 0.7% according to EFLM BV. Even if bias %=0 and CV %=0.5, the achievable sigma value is 1.4 and is considered unacceptable performance. Moreover, these values for bias% and CV% are too far away to be achieved in practical laboratory processes. Of course, parameters such as sodium must be controlled within a narrow range. Therefore, the targets of BV should be quite challenging but also realistic. In a study evaluating the process sigma performances of some clinical chemistry tests compared with laboratory data and manufacturer's brochure data, it was found that the goal of >3 sigma for laboratories cannot be achieved even when brochure data for parameters such as sodium, chloride, and calcium are used [12]. Manufacturers obtain precision under optimal conditions and bias from method comparison studies. Therefore, the data reported in the package insert may be considered quite optimistic compared with data obtained in routine laboratory practice [12]. For laboratory end users, trying to meet BV quality targets with current technologies is a waste of time, effort, and resources [12, 14]. In our opinion, many tests in our study had very low sigma values due to these very stringent criteria.

Although it seems reasonable to use the same TEa source for each test, it may be appropriate to use different TEa sources depending on the suitability for the test or the experience of the laboratory. Some TEa targets are quite generous and lead to a quality result of misleading optimism. Others, however, lead to a more pessimistic representation of quality performance, as in the case of BV. In the current situation, it is at the discretion of the laboratory director to choose the appropriate TEa criterion by making the most practical and appropriate decision.

Conclusion

The process sigma values reported in this study reflect the time period in which the data were collected and thus represent a "snapshot" of sorts. Of course, performance can fluctuate for many reasons, including temperature, pH, and variations between different reagents. Regular calculation of the process sigma would be more useful in terms of testing and continuous monitoring of instrument quality. This is because when we look at the data from our study and other studies presented in the literature, test performance may increase or decrease due to changes in the process. Accordingly, process sigma can be used as a suitable quality assurance tool to determine test quality and monitor quality changes on a regular basis.

Ethics Committee Approval: The study was approved by The Karadeniz Technical University Faculty of Medicine Clinical Research Ethics Committee (No: 2014-168, Date: 27/03/2015).

Authorship Contributions: Concept – M.E., S.C.K., A.O.; Design – M.E., S.C.K., A.O.; Supervision – M.E., S.C.K., A.O.; Funding – M.E., Y.A., A.O; Materials – M.E., Y.A.; Data collection &/or processing – M.E., S.C.K., A.O., Y.A., H.B.Y.; Analysis and/or interpretation – M.E., S.C.K., H.B.Y.; Literature search – M.E., H.B.Y.; Writing – M.E., H.B.Y.; Critical review – M.E., H.B.Y., A.O., S.C.K., Y.A.

Conflict of Interest: The authors declare that there is no conflict of interest.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

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