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Sigma metrics – a good quality control guide to assess analytical performance of a clinical chemistry laboratory

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ABSTRACT

Background: Clinical laboratories function to deliver accurate, reliable, and timely reported results which are used in decision-making, diagnosis, and monitoring. Sigma metrics help to assess analytic methodologies and provide benchmarks for laboratories to design protocols for internal quality control (IQC), address poor assay performance, and assess the efficiency of existing laboratory processes. Thus, this study was undertaken to estimate the coefficient of variation (CV%), bias%, and total allowable error (TEa) of quality control (QC) samples using EM 200, to compare the TEa using Clinical Laboratories Improvement Act (CLIA) guidelines, and to analyze the sigma metrics of level 1 QC samples. **Aims and Objectives:** The aims and objectives of the study are to estimate the CV%, Bias%, TEa of QC samples using EM 200, to compare the TEa using CLIA guidelines, and to analyze the sigma metrics of level 1 QC samples. **Materials and Methods:** A cross-sectional study was carried out in the Central Biochemistry Lab, Karwar Institute of Medical Sciences, Karwar. IQC data level 1 of 15 analytes was analyzed using EM200 for 3 months. CV% is calculated from internal quality data, whereas bias% is obtained from an external quality assurance program. Sigma metrics were calculated using bias% and CV%. TEa was calculated and compared with CLIA guidelines. **Results:** We have < 3 sigma values (unstable, unacceptable) for urea, creatinine, BID, serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase (SGPT), protein, cholesterol, calcium, 3–6 (ideal) for glucose, uric acid, total bilirubin, alkaline phosphatase, albumin, high-density lipoprotein, and >6 (excellent) for triglycerides. TEa observed less than or close to CLIA suggests quality requirement met, and TEa observed more than CLIA (urea, creatinine, BID, SGPT, protein, and calcium) suggests methodologies need evaluation. **Conclusion:** Sigma metrics help to assess analytical methodologies and augment laboratory performance. Each and every laboratory can use sigma metrics as a guideline for QC strategy and a self-assessment tool for proper functioning.

Key words: Quality control; Sigma metrics; Internal quality control; Total allowable error

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INTRODUCTION

Clinical laboratories play an important role in diagnosis and treatment by providing timely laboratory results. Accurate and precise results are very crucial, which help physicians and patients to take timely decision, proper management, and screening.[1] Quality control (QC) strategies are very important to identify analytical errors when a measurement procedure may not be providing results that are suitable for the use of laboratory results for medical decisions. Various practices are adopted to improve quality which include Levey-Jennings charts, following Westgard rules, and recording the coefficient of variation (CV%) for internal QC (IQC) purposes.[2] The laboratory testing process can be divided into three stages: pre-analytical stage, the analytical stage, and post- analytical stage. The pre-analytical stage

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contains two sub-stages outside the laboratory and within the laboratory.[3] Errors can happen at any of the above stages due to human intervention. Medical laboratory technicians are trained to rerun the QC samples till it reaches the acceptable limit only after which patient samples are run. To further assure quality, external quality assurance (EQA) programs are established and Z score or standard deviation index is calculated. These tools allow estimation of precision by minimizing random errors and ensuring accuracy by reducing bias. The backbone of a good laboratory rests on the QC program adopted by laboratories.

Bill Smith, the father of Six Sigma, decided to measure defects per million. Total quality management had become popular by the early 1990s. "PDCA" (plan, do, check, and act) is the model adopted for total quality management.[4-6] Six Sigma methodology was developed by Motorola, which aims to reduce cost, eliminate defects, and decrease variability during processing. It comprises of five steps: Define, measure, analyze, improve, and control. The sigma value indicates how often errors are likely to happen, the higher the sigma value, the less likely it is to occur errors in the laboratory performance and to produce false test results. The Six Sigma model includes an additional step, control, which helps to prevent defects from returning to the process. 1 sigma represents 6,90,000 errors/million reports, 2 sigma represents 3,08,000 errors/million reports, 3 sigma represents 66,800 errors/million reports, 4 sigma represents 6210 errors/million reports, 5 sigma represents 230 errors/million reports, and 6 sigma represents 3.4 errors/million reports.[7-9] Based on sigma analytical performance, it is classified into the following categories: >6: world class performance; 5-6:– excellent; 4–5: good; 3–4: acceptable; 2–3: poor; and <2: unacceptable.[10] The aim of our study was to study the sigma metrics of biochemistry analytes to improve the QC, evaluate the functioning of the instrument, and check the adequacy of the methodology being followed.

Aims and objectives

To estimate the CV%, Bias%, TEa of QC samples using EM 200, to compare the TEa using CLIA guidelines, and to analyze the sigma metrics of level 1 QC samples

To study the sigma metrics of biochemistry analytes to improve the QC, evaluate the functioning of the instrument,and check the adequacy of the methodology being followed

MATERIALS AND METHODS

A cross-sectional study was carried out in the Central Biochemistry Lab, Karwar Institute of Medical Sciences, Karwar. We analyzed sigma metrics for 15 parameters with the automated chemistry analyzer EM-200. The study protocol was approved by institutional human ethics committee (Proposal No. IEC/KRIMS/21/2023-24).

IQC data (level 1) of 15 analytes were analyzed prospectively over a period of 3 months from July 2023 to September 2023 with EM-200, and external QC material is obtained from CMC Vellore, which is run monthly as a part of the EQA scheme in EM-200. Normal (L1) levels of QC materials were routinely assayed in our laboratory before reporting patient samples every day. The instruments were calibrated regularly. The analytes assessed were glucose, urea, creatinine, direct bilirubin, indirect bilirubin, serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), albumin, protein, uric acid, cholesterol, triglycerides, high density lipoprotein (HDL), and calcium.

The sigma value was calculated with the following formulas:

Total allowable error (TEa): The total allowable difference from the accepted reference value is seen in the deviation of a single measurement from the target value. The TEa observed in our laboratory was calculated using the formula

$$\text{TEa observed} = \text{Bias} + \%\ \text{CV} \times 2$$

The observed TEa is compared with that obtained by Clinical Laboratories Improvement Act (CLIA) guidelines.

Bias: Bias is the systemic difference between the expected results obtained by the laboratory's test method and the results.

$$\text{Bias}\ \% = \frac{\begin{array}{l}\text{Mean of all laboratories}\\ \text{using same instrument}\\ \text{and method} - \text{our mean}\end{array}}{\begin{array}{l}\text{Mean of all laboratories}\\ \text{using same instrument}\\ \text{and method}\end{array}} \times 100$$

CV% is the analytical value (CV%) of the test method. The coefficient of variance (CV) was calculated as follows:

$$\text{CV}\ \% = \frac{\text{Standard deviation}}{\text{Laboratory Mean}} \times 100$$

Sigma metrics are calculated from CV, percentage bias, and TEa for parameters by the following formula:

$$\text{Sigma} = (\text{TEa} - \text{Bias})/\text{CV}$$

Quality Goal Index (QGI) ratio: QGI is characterized by the relative extend to which both bias and precision meet their respective goals. This was used to analyze the reason

for lower sigma in analytes, i.e., whether the problem is due to imprecision, inaccuracy, or both (Table 1).

$$QGI = Bias/1.5 \times CV\%$$

The standard deviation (SD) quantifies how close numerical values are in relation to each other, and it increases as the concentration of the analyte increases. CV is the ratio and standardization of SD that allows comparison of variability regardless of analyte concentration (which does not vary with changes in measurement units). Precision is the closeness of agreement between independent, repeated results obtained from the same sample under specific conditions, and therefore, CV is a measurement of precision. Less CV means the better precision. Bias is difference between measured and actual value. It is used to describe inaccuracy of method; lower the bias more is accuracy.

Figure 1 shows normalized sigma method decision chart for biochemical analytes.

The normalized sigma method decision charts are derived from the website https://www.westgard.com/normalized-opspecs- calculator. htm. TEa, bias, and CV values are given, and a normalized sigma method decision chart is made which shows the analytical performance of each analyte, where abscissa represents CV/TEa% and ordinate represents Bias/TEa%. The chart was divided into six different regions by five differently colored lines; each region represents the level of analytical performance of each analyte. The sigma values from bottom left to top right represented sigma >6, 6> sigma ≥5, 5>sigma ≥4, 4>sigma > 3, 3> sigma ≥2, and sigma <2, respectively.[11]

Table 1: Quality goal index and reason for lower sigma values

QGI	Problem
<0.8	Imprecision
0.8–1.2	Imprecision and inaccuracy
>1.2	Inaccuracy

QGI: Quality goal index

RESULTS

Table 2 shows the average CV% of all parameters in level 1, and Tables 3 and 4 show the average CV%, average bias%, sigma and QGI, and comparison of TEa with CLIA guidelines. We have <3 sigma values (unstable, unacceptable) for urea, creatinine, BID, SGOT, serum glutamate pyruvate transaminase (SGPT), protein, cholesterol, calcium, 3–6 (ideal) for glucose, uric acid, total bilirubin, alkaline phosphatase (ALP), albumin, HDL, and >6 (excellent) for triglycerides. TEa observed less than or close to that of CLIA guidelines suggests that quality requirements are met, and TEa observed more than CLIA (urea, creatinine, BID, SGPT, protein, and calcium) suggests that methodologies need evaluation. We have observed average bias % <3 for 9 analytes (glucose, urea, creatinine, uric acid, BIT, protein, albumin, cholesterol, and triglycerides), 3.1–6 for 4 analytes (SGOT, ALP, HDL, and calcium), >6 for 2 analytes (BID, SGPT), average CV% <3 for 2 analytes (glucose, albumin), 3.1–6 for 9 analytes (urea, uric acid, BIT, BID, SGPT, cholesterol, TG, HDL, and calcium), and >6 for 4 analytes (creatinine, protein, SGOT, and ALP).

Decisions are taken according to the Westgard Sigma Flow Chart (Figure 2).

DISCUSSION

The importance of quality management system implementation in clinical laboratories is to provide test result with utmost quality which is used for disease screening, diagnosis, monitoring, and treatment.[12] Pre-analytical, analytical, and post-analytical processes should be continuously verified using internal and external audits, which is important to maintain the QC process.[13] An individualized QC plan protocol based on sigma values obtained from

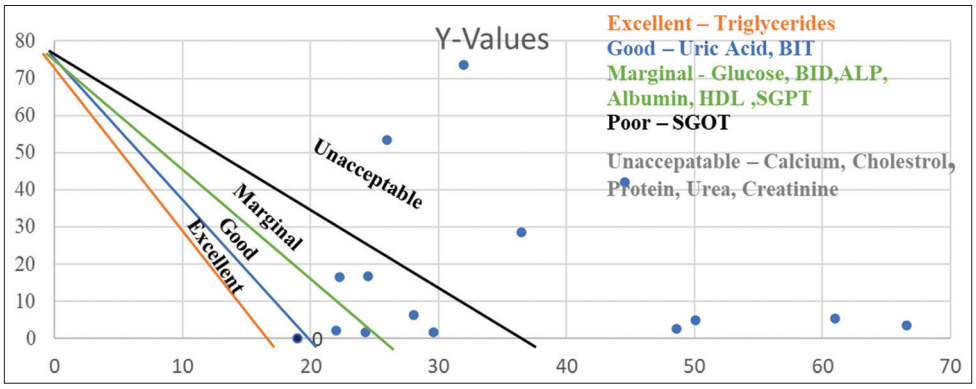

Figure 1: Normalized method decision chart for 15 biochemistry analytes

Table 2: CV% of all 15 analytes during study period for level 1

Analytes	July	August	September	Avg CV%
Glucose	3.7	1.85	2.9	2.81
Urea	4.35	10.2	3.8	6.11
Creatinine	9.2	6.9	5.8	7.3
Uric acid	4.8	3.8	3.8	4.13
BIT	5.5	2.7	5	4.4
BID	9	4.2	6.2	6.46
SGOT	6.5	11	4.6	7.36
SGPT	5.3	3.5	7	5.26
ALP	7.6	6.7	6	6.76
Protein	8.7	8.3	3	6.66
Albumin	3.5	1.98	3.41	2.96
CHOL	5.6	4.43	5	5.01
TG	4.2	3.33	4.1	3.87
HDL	5	1.04	8.7	4.91
Calcium	5.69	4.6	4.4	4.89

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, TG: Triglyceride, HDL: High-density lipoprotein, CHOL: Cholesterol, CV: Coefficient of variation

Table 3: Bias %, TEa, sigma metrics for level 1 QC

Analytes	AVG CV %	AVG Bias %	QGI	AVG Sigma
Glucose	2.81	0.64	1.19	3.33
Urea	6.11	0.53	2.15	1.54
Creatinine	7.3	0.39	1.8	2
Uric acid	4.13	0.28	0.77	4
BIT	4.4	0.44	1.2	4.44
BID	6.46	14.74	4.32	0.81
SGOT	7.36	5.75	2.79	1.93
SGPT	5.26	10.7	3.7	1.76
ALP	6.76	5	2.2	3.69
Protein	6.66	0.35	1.54	1.44
Albumin	2.96	0.15	0.29	3.32
CHOL	5.01	0.49	1.63	1.9
TG	3.87	0.39	1	6.35
HDL	4.91	3.34	10.93	5.4
Calcium	4.89	4.63	14.8	1.3

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, CHOL: Cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, TEa: Total allowable error, QC: Quality control, CV: Coefficient of variation, QGI: Quality goal index

sigma metric analysis should be designed by every individual laboratory as part of good laboratory practice.[14] Sigma metrics are an excellent tool to predict instrument quality and are a pointer to tests that require minimal QC rules to monitor the performance of the method.[15]

In our study, we analyzed 15 analytes over a period of 3 months (July 2023–August 2023) and assessed for sigma metrics. We have calculated CV%, Bias%, Sigma, QGI, and TEa for 15 analytes using level 1 QC samples using EM-200. In our study, we obtained higher CV values for SGOT, creatinine, ALP, and protein and they are correlated to precision. Lesser CV means better precision, which indicates that the above parameters' precision is low.

Table 4: Comparison of observed TEa to CLIA guidelines

Analytes	TEa observed	TEa as per CLIA
Glucose	6.24	10
Urea	12.75	10
Creatinine	14.99	15
Uric acid	8.54	17
BIT	9.24	20
BID	27.54	20
SGOT	20.4	20
SGPT	21.1	20
ALP	18.52	30
Protein	13.67	10
Albumin	6.07	10
CHOL	10.51	10
TG	8.13	25
HDL	13.16	20
Calcium	14.41	11

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, CHOL: Cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, TEa: Total allowable error, CLIA: Clinical Laboratories Improvement Act

Bias is the difference between the measured result and the actual value, used to describe the inaccuracy of the method. In our study, we obtained a higher bias for ALP and direct bilirubin. The lower the bias more is the greater the accuracy, which suggests that these parameters have chances of inaccuracy in the method of measurement which need further evaluation.

Sigma metrics calculated and our study obtained < 3 sigma values (unstable, unacceptable) is for urea, creatinine, BID, SGOT, SGPT, protein, cholesterol, calcium, 3–6 (ideal) for glucose, uric acid, total bilirubin, ALP, albumin, HDL, and >6 (excellent) for triglycerides. The QGI ratio for these parameters with sigma <3 depicts the problem occurring in level 1 QC due to inaccuracy (QGI >1.2).[15]

Similar studies were done, and different sigma metrics were reported. Adiga et al., reported seven parameters (urea, ALT, BD, BT, Ca, and creatinine) in L1 with <3 sigma in XL-640.[3] Koshy and Raza reported <3 sigma for urea.[16] Sigma values for creatinine and urea were reported between 3 and 6 by Nanda and Ray and >6 sigma by Singh et al., for creatinine. Variations in sigma values between our study and others in a few analytes can be due to differences in the instrument used, calibrators used, QC material used, other pre- and post-analytical conditions, reagents, bias calculations, and varying EQUAS providers.

Evaluating and calculating the sigma metric are important in designing and implementing QC strategies.

Based on sigma values, QC can be tailored as follows: (Figure 2)

1. 6 sigma (excellent performance): IQC can be run once

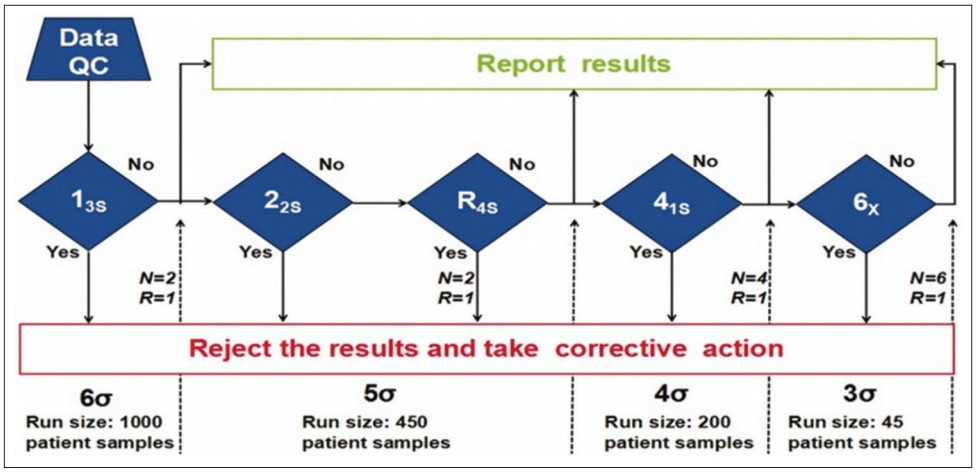

Figure 2: Westgard sigma flow chart with batch size Sigma = (TEa-Bias)/CV%. Sigma values of each analyte calculated based on above sigma formula. Then, according to flow chart at bottom of sigma scale, the corresponding QC rules, the number of IQC products, and the batch size for analysis were selected as IQC scheme. "N" represents number of QC materials and "R" represents number of analytical batch. "Yes" indicates a violation of quality control rules, in this case result was rejected and corrective action was taken. "No" indicates no violation of the quality control rules, in this case, results were accepted and reported. IQC: Internal quality control, QC: Quality control, TEa: Total allowable error, CV: Coefficient of variation

per day with one level and follow the 1_{3s} rule
2. 4 sigma–6 sigma (suited to purpose): IQC can be run once per day with two levels per day and follow a single IQC rule
3. 3 sigma–4 sigma (poor performers): IQC can be run twice per day with two levels of IQC per day and use a multi-rule system
4. <3 sigma (problematic): IQC runs three times per day with three levels; consider testing in duplicate; and use the maximum IQC rules.

With a sigma scale of <3, root cause analysis based on five vital aspects: manual intervention, equipment, materials, method, and environment, found out before the method can be routinely used for releasing the results.[17] A quality improvement plan based on personnel proficiency, the use of alternative methods, and the change of reagents can be done for poor sigma performance analytes. Improvement measurements should be taken by giving proper staff training and further assessment, instrument maintenance, reagent selection and evaluation, monitoring lot-to-lot reagent changes, detection of system performance evaluation, calibration of analyte and calibrate verification, and a maintaining proper environment (temperature and humidity monitoring), which can improve the analytical performance, which in turn improves the laboratory performance. We have also calculated TEa for all 15 analytes and compared them with CLIA guidelines (Table 4). TEa observed <TEa (CLIA) or close to is considered quality requirement met and an instrument suitable for measurement analyte. Analytes for which TEa observed >TEa (CLIA) were urea, direct bilirubin, SGOT, SGPT, cholesterol, and calcium (L1), suggesting that respective methodologies need further evaluation. We have derived the QC strategy for the analytical performance of analytes from our sigma values while TEa values assure us to use the correct methodologies for analytes showing poor performance.

Limitations of the study

Study was done only using level 1 quality control samples which are running routinely in our lab.

CONCLUSION

Clinical laboratories aim to provide accurate and reliable test results which are very important for the timely diagnosis and treatment of diseases. To assess the analytical methodologies and improve laboratory performance, Sigma metrics can be used as a good tool for QC. Application of six Sigma helps to reduce process variability, to quantitate the approximate number of analytical errors, and evaluate and guide better QC practices. For analytes scored poor sigma metrics (<3), strict monitoring and modification of the QC procedure with a change in method should be adopted to improve the laboratory performance.

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Authors' Contribution:
LRC -Prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **PRT**- Concept, design, manuscript preparation, editing, and manuscript revision; **MBN** -Design of study, preparation of figures, coordination, statistical analysis, interpretation, and manuscript revision.

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