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Original Article

Analytical Perfomance Assessment in Biochemistry Laboratories Using Six Sigma

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ABSTRACT

Background: This study was aimed to evaluate analytical quality in clinical Biochemistry laboratory through six sigma. It provides quantitative framework for process performance assessment and facilitates objective data driven identification of process variability and opportunities for continuous quality improvement. **Materials And Methods**: In the present study, internal and external quality control data were analyzed retrospectively for the period from September 2025 to January 2026. Laboratory mean, standard deviation, coefficient of variation and bias were calculated for the selected parameters. Sigma was calculated for level I & II of internal quality control (IQC). **Results**: For Biochemistry Parameters, TP, Albumin, Urea, Creatinine, Glucose, Cholesterol, Triglyceride had <3 sigma for both Level 1 and 2 while CK, Amylase, HDL, Phosphorus, Magnesium, Iron, ALP, ALT has sigma value between 3-6 for both Level 1 and 2. Total bilirubin, UA and AST had sigma value <3 for L1 and sigma value between 3-6 for L2. Immunology parameters included in our study were TSH,TT3, FT3, FT4, TT4 in period between January to December 2025. Satisfactory sigma value was obtained for TSH and TT3 between 3-6 while TT4, FT3 and FT4 performed poorly with sigma <3.
Conclusion: Six Sigma in laboratories enhances analytical accuracy, reduces variability, and improves patient outcomes through data-driven quality control. It is cost-effective long term by minimizing errors, repeats, and wastage.

Keywords: Quality control, Analytical Quality, cost-effective, Six sigma.

INTRODUCTION

Clinical laboratories are operated and governed primarily through the principles of quality management, quality assurance, and quality control.[1] The quality of laboratory is an emerging concern worldwide because of incremental clinical value that laboratory tests hold for screening, diagnosing, prognosticating and therapeutic monitoring of most human diseases. As Laboratory medicine will remain vital for patient safety and patient flow, maintaining and improving quality of laboratory is crucial.[2]

Total quality management framework for management of quality in healthcare laboratory includes Quality control(QC), Quality assessment(QA), Quality improvement (QI), Quality Planning (QP)and Quality laboratory process(QLP).[3] After diagnosis-related groups and prospective reimbursement systems were introduced, greater attention was given to cost control and efficient laboratory operations while maintaining quality of reports.[4] Six sigma process control is an evolution in quality management that is being widely implemented in business and industries. Six Sigma quality management has gained worldwide recognition since its creation by Bill Smith of Motorola in 1986 [5]

Six Sigma is derived from a Greek symbol used to represent variation within a process. In this method, quality is evaluated by measuring the number of defects per unit produced. The sigma level shows how often errors happen—when the sigma value is high, defects are rare, and when it is low, defects occur more frequently. A process is regarded as achieving "world-class" quality when it operates at the Six Sigma level, which equals approximately 3.4 defects per million opportunities (

Table 1). [6, 7]In the context of healthcare, striving toward Six Sigma performance is critical due to the extensive utilization of medical services across large populations. Given the high volume of daily clinical and laboratory processes, even minimal error rates can translate into a substantial absolute number of defects. Moreover, minor deviations in healthcare delivery may result in significant or potentially life-threatening consequences for patient outcomes, underscoring the necessity for stringent quality control and continuous process improvement. [8]

In 2001, Nevelainen et al. introduced the first benchmark of laboratory quality based on the sigma scale. [9] Since then six sigma tool have been used by laboratories to check method quality, QC optimization, change the number of rules and controls run and to change the frequency of QC. According to Nitin at al. the application of six sigma methodology in laboratory has significant role to minimize both variance and quality control processes to improve the compliance with the vital specifications.[10] Along with laboratory, the processes involved in the health industry also includes timeliness, patient satisfaction and the efficiency of physicians. Cherry and Seshadri showed that implementation of Six Sigma could also benefit the emergency department, admissions, invoicing and surgery documentation processes.[11]

Unlike other quality initiatives borrowed by the health care sector from the industrial sectors like the Total Quality Management (TQM) and critical-to-quality(CTQ), Six sigma is different in that the improvement obtained through this approach provides sustained strategic achievements with long-lasting benefits. Six Sigma programs also include powerful methods like Define-Measure-Analyze-Improve-Control (DMAIC) and root cause analysis to identify and remove defects and reduce process variation. [12] So , as part of a quality improvement initiative, the present study was undertaken to evaluate sigma metrics for multiple parameters within the clinical biochemistry laboratory. These metrics were utilized as an objective self-assessment tool to critically analyze the existing quality control (QC) strategies employed in the clinical chemistry laboratory and to facilitate the optimization of QC frequency and protocols based on analytical performance.

Table 1: Levels of sigma performance and corresponding Defects per Million Opportunities.

Sigma Level	DPMO*
6	3.4
5	233
4	6,210
3	66,807
2	308,537
1	690,000

MATERIAL AND METHODS

Study type: Retrospective study

Data collection

Study data were extracted during September 2025 to January 2026 from Clinical Biochemistry Laboratory, Narendra Modi Medical College and LG General Hospital, Maninagar, Ahmedabad. Internal Quality Control(IQC) materials were obtained from Bio-Rad, USA and external quality control data was obtained by participating in External Quality Assurance scheme (EQAS) of Bio-Rad. According **to** ISO 15189:2022 - 7.3.7. Both levels of biochemisty QC materials level I & II were run assayed before running patient samples. To ensure the stability of the method at least one control is run every eight hours.For immunology parameters IQC and EQAS material was obtained from Biorad. QC data obtained from Abott ci 8200 and validated through strict, documented statistical rules (e.g., Westgard) to monitor performance and minimize clinical risk.

The data obtained for the study are coefficient of variation percent (CV%) from internal quality data and Bias (%) from External Quality Assurance Scheme (EQAS). Various biochemistry parameters included in the study are Total Protein (TP, Biuret method), Albumin (Bromcresol Green (BCG) method), Total Bilirubin (Diazonium Ion method), Urea (Urease, UV method) , Creatinine (Alkaline picrate method), Uric Acid (UA, Uricase method), Glucose (Hexokinase method), Creatinine Kinase (NAC activated), Cholesterol (Cholesterol oxidase, esterase, peroxidase method), Triglyceride (Enzymatic, end point method), HDL(Direct measure, polymer−polyanion method), Phosphorus (Phosphomolybdate method), Magnesium (Enzymatic method), iron (Ferene method), Amylase (G7 PNP, Blocked method), Alkaline phosphatase (ALP, PNPP, AMP Buffer method), alanine aminotransferase (ALT or SGPT) (UV with P5P method), aspartate aminotransferase (AST or SGOT) (UV without P5P method). Immunology parameters included in study are TSH, TT3, TT4, FT3, FT4 (all done by chemiluminescence method)

Sigma value was calculated with the following formulas: Total allowable error (TEa): It is the total allowable difference from accepted reference value seen in the deviation of single measurement from the target value. TEa values of various

parameters were taken from Clinical Laboratories Improvement Act (CLIA) guidelines (January, 2025) except for FT3 Rilibak guidelines used.

Bias: Bias is the systematic difference between the expected results obtained by the laboratory's test method and the results that would be obtained from an accepted reference method. Bias was derived as follows:

$$\text{Bias (\%)} = \frac{\text{Mean of all laboratories using same instruments and methods} - \text{Our mean}}{\text{Mean of all laboratories using same instrument and method}} \times 100$$

Coefficient of variance (CV): CV% is the analytical coefficient of variation of the test method. CV is calculated as follows:

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

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

$$\Sigma(\sigma) = (\text{TEa} - \text{bias})/\text{CV\%}$$

RESULTS

The present study analyzed the 18 biochemical analytes and 5 immunology parameters and interpreted using sigma metrics. The investigator have calculated mean, SD, CV%, Bias% and sigma values for each parameter at level I and level II for biochemistry parameters and level I and level II and level III for immunology parameters . CV% was calculated from internal QC data for each parameter and Bias% was calculated from EQAS data. TEa was taken from CLIA guidelines except for FT3 Rilibek guideline was used. With these data sigma value was calculated using the formula mentioned in methodology. For various biochemistry parameters TEa, Bias% and CV% used to calculate six sigma as shown in Table 2.

Table 2: TEa%, Bias%, CV% and Sigma metrics for different biochemistry parameters for Level I (L1)&Level 2(L2)

No	Parameter	Total Allowable Error [TEa (%)]	Average Bias %	Level 1		Level 2	
				Average CV%(L1)	Sigma (L1)	Average CV%(L2)	Sigma (L2)
1.	**TP**	**8**	**0.03**	**3.88**	**2.05**	**3.54**	**2.25**
2.	**Albumin**	**8**	**0.65**	**3.47**	**2.12**	**3.44**	**2.14**
3.	**T bili**	**20**	**5.2**	**5.04**	**2.94**	**3.17**	**4.67**
4.	**Urea**	**9**	**2.84**	**5.25**	**1.17**	**4.19**	**1.47**
5.	**Creat**	**10**	**1.32**	**3.43**	**2.53**	**3.12**	**2.78**
6.	**UA**	**10**	**1.48**	**3.25**	**2.62**	**2.78**	**3.06**
7.	**Glucose**	**8**	**0.21**	**3.92**	**1.99**	**2.72**	**2.86**
8.	**CK**	**20**	**5.85**	**4.53**	**3.12**	**3.65**	**3.88**
9.	**Chol**	**10**	**1.98**	**4.27**	**1.88**	**4.19**	**1.91**
10.	**Trig**	**15**	**4.76**	**4.57**	**2.24**	**4.73**	**2.16**
11.	**HDL**	**20**	**1.43**	**4.27**	**4.35**	**4.97**	**3.74**
12.	**Phos**	**10**	**0.01**	**3.3**	**3.03**	**3.26**	**3.06**
13.	**Mag**	**15**	**1.65**	**4.32**	**3.09**	**3.35**	**3.99**
14.	**Iron**	**15**	**2.28**	**4.06**	**3.13**	**4.15**	**3.07**
15.	**Amy**	**10**	**1.36**	**2.76**	**3.13**	**2.9**	**3.0**
16.	**ALP**	**20**	**-3.12**	**5.83**	**3.97**	**4.94**	**4.68**
17.	**ALT**	**15**	**-6.08**	**6.56**	**3.21**	**4.39**	**4.80**
18.	**AST**	**15**	**4.21**	**3.85**	**2.80**	**3.31**	**3.26**

Table 3: Parameters with < 3 sigma value for L1 and L2

Parameter	<3 L1	<3 L2
Total Protein	2.05	2.25
Albumin	2.11	2.13
Urea	1.1	1.4
Creat	2.5	2.7

Glucose	1.9	2.8
Cholesterol	1.8	1.9
Triglyceride	2.2	2.1

Table 4: Parameters with sigma value 3-6 for L1 and L2

Parameter	3-6 L1	3-6 L2
CK	3.1	3.8
Amylase	3.1	3.0
HDL	4.3	3.7
Phosphorus	3.0	3.0
Magnesium	3.0	3.9
Iron	3.1	3.0
Alp	3.9	4.6
ALT	3.2	4.8

Table 5: Parameters with sigma value< 3for L1 and 3-6 for L2

Parameter	<3 L1	3-6 L2
Total bili	**2.94**	4.6
UA	**2.62**	3.0
AST	**2.80**	3.2

For Biochemistry Parameters, TP, Albumin, Urea, Creatinine, Glucose, Cholesterol, Triglyceride had <3 sigma for both Level 1 and 2 (Table 3) while CK, Amylase, HDL, Phosphorus, Magnesium, Iron, ALP, ALT has sigma value between 3-6 for both Level 1 and 2. (Table 4).Total bilirubin, UA and AST had sigma value <3 for L1 and sigma value between 3-6 for L2(Table 5)

Table 6: TEa%, Bias%, CV% and Sigma metrics for immunology parameters for Level I (L1)&Level 2(L2) & Level 3(L3)

No	Parameter	Total Allowable Error [TEa (%)]	Average Bias %	Level 1		Level 2		Level 3	
				Average CV%(L1)	Sigma (L1)	Average CV%(L2)	Sigma (L2)	Average CV%(L3)	Sigma (L3)
1.	TSH	20	1.43	5.78	3.71	6.51	3.29	5.95	3.60
2.	TT3	30	2.74	6.83	4.79	7.65	4.28	7.75	4.22
3.	TT4	20	2.30	8.48	2.09	9.14	1.94	11.75	1.51
4.	FT3	20	3.37	9.47	2.47	7.81	2.99	8.58	2.72
5.	FT4	15	2.65	4.97	2.49	8.62	1.43	13.07	0.95

Table7: Sigma metrics for immunology parameters for Level I (L1)&Level 2(L2) & Level 3(L3)

Parameter	Sigma L1	Sigma L2	Sigma L3
TSH	3.71	3.29	3.60
TT3	4.79	4.28	4.22
TT4	2.09	1.94	1.51
FT3	2.47	3.04	2.73
FT4	2.49	1.43	0.95

Immunology parameters included in our study were TSH,TT3, FT3, FT4, TT4 in period between January to December 2025.As per Table 6 CV% values of IQC for level I, II and III were found between 5 to 10 for all parameters. While Sigma value for TSH and TT3 was between 3-6 sigma which means 1 3S/2 2S/R 4S/4 1S rule should be used while TT4, FT3 and FT4 had sigma value <3.(Table 6 &7)

DISCUSSION

Quality control are paramount importance in Clinical laboratories and to maintain quality of patient reports laboratories are in a regular search of methods to solve pre analytical,analytical & post analytical problems and decrease errors to a very low level .Approximately 30-75% for preanalytical, 4-30% for analytical, and 9-55% for postanalytical phase errors

found [13].Six sigma focuses on reducing analytical errors. Criteria for quality includes in terms of sensitivity and specificity, accuracy (closeness to the true value), precision (reproducibility of a test result) etc. To achieve this, different levels of control materials for IQC is used and data is prepared by charts like Levey Jennings for precision. Violation of different westgard rules points towards a particular random or systematic errors[3]. Another external quality control (EQC) involves analyzing and reporting of control samples supplied by an external agency, at a predefined time interval of a fortnight or a month for accuracy. The external supplier of the QC sample studies for mean ,bias & Z score etc.[14]

Although external quality control (EQC) and internal quality control (IQC) are essential components of laboratory quality assurance, they are limited in their ability to quantitatively estimate the exact frequency of analytical errors or defects. In contrast, the application of sigma (σ) metrics provides a more robust and quantitative framework for assessing defect rates and overall analytical performance within the laboratory.

According to Cooper et al , guidelines published suggests grouping of tests as per sigma performance and QC strategy planned for cost effective manner as follows
>6σ (excellent tests) –one QC per day (alternating levels between days) and to check 13s rule.
4σ–6σ (suited for purpose) –two levels of QC per day and observe for 12.5s rule.
3σ–4σ (poor performers) –two levels of QC twice per day for combination of rules with
<3σ (problems) – three levels, , three times a day maximum QC, preferably testing specimens in duplicate. [15]
However Another easy guidelines for suggesting levels of QC as proposed by Westgard are as follows for Multirule combinations of Westgard rules though more powerful than single rule , when there is low sigma value
≥6σ :– with13.5s greater rule 2 levels of QC per day with
5σ :– with a 12.5s or 13s rule 2or 3 levels of QC per day
4σ :– with a 13s / 2 22s / R 4s / 4 1 s rule 3or 4 levels of QC per day
3.5σ :– with a 13s / 2 22s / R 4s / 4 1 s rule 6 of QC per day
<3.5 σ :– with a 13s / 2 22s / R 4s / 4 1 s rule maximum affordable levels of QC per day.[16]

So far only few guidelines published for QC measurements related to calibration , manufacturer maintenances, IQC evaluation for different analytical system. It is crucial that an analytical procedure should achieve a higher sigma level if a for reliability is to be attached to the results [17]

In Our Study out of total 18 biochemistry parameters sigma parameters like CK, Amylase, HDL, Phosphorus, Magnesium, Iron, ALP, ALT, were in 3-6 σ(Table 4) So, 1 3S/2 2S/R 4S/4 1S rule should be used for the quality. In one study done Adiga US et al Glucose, AST, cholesterol, uric acid, total protein (L1) and ALT, cholesterol, creatinine and glucose (L2) had sigma value between 3-6 which is similar for some parameters as our study.[18]

For biochemistry parameters like Total Protein, Albumin, Urea, Creatinine, Glucose, Cholesterol, Triglyceride were identified with sigma values (< 3 σ) for both level 1 and 2 (Table 4). Total bilirubin, UA and AST had sigma value <3 for L1 and sigma value between 3-6 for L2(Table 5) High imprecision was observed with high CV percentage observed for this parameters which indicates that stringent actions should be taken to improve performance of these parameters. Study done by Afrifa et al., in 2015 had similar finding of low sigma value for Total Protein, Albumin, Urea, Creatinine, Glucose, Cholesterol, Triglyceride.[19] Similar studies were done by Nitin C, Thomas V, Aggarwal KV etc., for use of sigma metrics for quality assessment of laboratory. [10, 20,21] Variations in sigma values between our study and others can be attributed to the difference in the instrument used, quality control material used and other pre & post analytical conditions.

Sigma value for triglyceride, HDL, AST, ALT was shown higher than 6, in a study by Bhawna Singh et al.[22] in contrast our sigma value between 3 to 6 . In our study, the sigma metrics value for Creatinine was found <3 , in contrast to that reported by Carl Garber (sigma value for creatinine 6).[23]

Thyroid profile parameters included in our study were TSH,TT3, FT3, FT4, TT4 in period between January to December 2025.As per Table 5 CV% values of IQC for level I, II and III were found between 5 to 10 for all parameters. While Sigma value for TSH and TT3 was between 3-6 sigma which means 1 3S/2 2S/R 4S/4 1S rule should be used while TT4, FT3 and FT4 had sigma value <3. So, for these parameters, the frequency of IQC should be detailed and elaborate action plans required to maintain quality control.

The sigma values were found to be higher than 3 for TSH for all 3 levels which is consistent finding with study done by Gulbahar O et al., Smita V et al., and Saurabh Singh [24 25, 26]

In another study performed by Nar R and Emekli DI, observed that sigma value of TSH was >6 for QC for three months which is showing excellent performance, [27] which is opposite to our study.

Not a single parameter in our study had greater than 5 sigma value so existing QC protocol needs to be changed. Difference between our statistical data and others study like E Xuehui Mao et al [28],and Thomas V et al [29] were due to the in variation

in QC samples as well as instrument , reagents and their integrity, method of analysis, different IQC and Calibrator material, difference in bias calculated due to different proficiency testing bodies.

CONCLUSION

Quality means conformance to the requirements of the end users. Quality control is ongoing process in laboratory to improve patient reports. So for accuracy and precision of any lab result not only mean, SD, CV% and Bias% of the any analyte should be monitored but sigma matrix could also be meticulosly applied for cost effectiveness. The analytical performance of our laboratory According to six sigma methodology, it was acceptable for TSH, TT3 , CK, Amylase, HDL, Phosphorus, Magnesium, Iron, Alkp, ALT with 3-6 σ . However unsatisfactory value for FT3,FT4, ,TT4, Total Protein, Albumin, Urea, Creatinine, Glucose, Cholesterol, Triglyceride, Total bilirubin, UA, AST which shows instability and low consistency of results being delivered which require proper root cause assessment of analytical process. Laboratory should use alternate methods and reagents for analytical quality to maintain high sigma metrics. A laboratory with high sigma metrics could have low turn time with excellent quality.

Limitation(s)

Limitation of the present study was done for single company & instrument.It can be upgraded to various company and instrument for better methodologies, Along with pre-analytical, analytical and post-analytical processes which may help in reducing the errors and improve the sigma values in the laboratory evaluation of general performance of clinical laboratory.

ETHICAL DECLARATIONS

Review or approval by an ethical committee was not required for this study we used existing data from quality control run in machine and did not involve any studies using human samples but Head of Department permission taken.

Credit authorship contribution statement

Dr Sahema Shaikh: Writing – original draft, Methodology, Formal analysis, Conceptualization.
Dr Usha Patel: Writing – review & editing, Methodology, Formal analysis, Data
Dr Mahammad Shaahid Mansuri, Dr Bharvi Pandya and Dr Mahesh Madole **:** Editing

Conflict of interest

All authors declare that they have no Conflict of interest for financial or personal relationships that could have appeared to influence the work reported in this paper.

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