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SIGMA METRICS-BASED ASSESSMENT OF ANALYTICAL QUALITY IN ROUTINE ANALYTES OF CLINICAL BIOCHEMISTRY LABORATORY: A PROSPECTIVE DUAL-LEVEL INTERNAL QUALITY CONTROL STUDY

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ABSTRAC

Background: Laboratory investigations inform over 70% of clinical decisions, placing analytical performance at the centre of quality patient care. Six Sigma analysis converts routine imprecision and bias measurements into a quantitative performance metric, allowing laboratories to compare their results against established quality goals and to select appropriate quality control (QC) strategies. This study applied Six Sigma metrics and the Quality Goal Index (QGI) to 21 routine biochemistry analytes at two QC concentration levels over a six-month period at a teaching hospital in Maharashtra.

Materials and Methods: A prospective observational study was carried out between October 2025 and March 2026. Imprecision was captured as %CV from two commercial internal quality control (IQC) materials — Level 1 (normal) and Level 2 (pathological). For Level 1, bias was derived from monthly External Quality Assessment Scheme (EQAS) peer-group comparisons; for Level 2, IQC-based bias specific to the pathological concentration range was used. Sigma scores were computed as: $\sigma = (TEa - Bias\%) / \%CV$ using TEa values from the Ricos biological variation database and CLIA regulatory limits. QGI was computed at both levels.

Results: Sigma scores at Level 1 ranged from 2.29 (UIBC) to 15.00 (Amylase). Ten of 21 analytes (47.6%) were World Class ($\sigma \geq 6$), five were Excellent (σ 5–6), five were Good or Marginal (σ 3–5), and UIBC alone was Unacceptable ($\sigma < 3$). At Level 2 (18 evaluable analytes), scores extended from 2.92 (UIBC) to 17.39 (ALP); eleven analytes (61.1%) achieved World Class status. QGI analysis classified 17 of 21 analytes as imprecision-dominant at Level 1.

Conclusions: Analytical quality was satisfactory for most tests. UIBC requires urgent process review. Evaluating sigma performance across two QC concentration levels exposes quality differences that a single-level strategy would miss. Sigma-stratified QC protocols would allow better allocation of laboratory resources.

Keywords: Six Sigma; sigma metrics; internal quality control; quality goal index; clinical biochemistry; EQAS; total allowable error; Westgard rules; dual-level IQC; root cause analysis.

INTRODUCTION

Accurate and reproducible laboratory results are indispensable to safe clinical practice. Published estimates indicate that laboratory data influence more than 70% of all diagnostic and therapeutic decisions,[1] which means that even modest analytical errors can translate into genuine clinical harm — delayed diagnoses, incorrect drug dosing, or unnecessary investigations. Of the three phases of the total testing process, the analytical phase is the most directly controllable by the laboratory and thus demands structured quality management as both a professional obligation and a regulatory requirement.

Conventional quality control methods — Levey-Jennings charts and the Westgard multirule system — detect analytical failures on a run-by-run basis by comparing QC measurements against fixed statistical limits.[2] These approaches have served laboratories well for decades; however, they do not provide an overall measure of how well a method is performing relative to the clinical needs of that test. A method that barely passes the 1_3 s rule every day may still be delivering results with clinically unacceptable total error. Six Sigma methodology addresses this gap by placing analytical performance in the context of the clinical quality requirement.[1,4] Originally developed in the manufacturing sector by Motorola and later adapted for clinical laboratories by Westgard and colleagues,[3,4] sigma metrics measure how much of the allowable error is used by actual analytical error. This helps determine whether QC frequency can be safely reduced for well-performing methods and identifies processes that require urgent improvement.[4]

The sigma (σ) score is calculated from three parameters: the Total Allowable Error (TEa), the method bias, and the imprecision (%CV), according to the formula:

$$\sigma = (TEa - Bias\%) \div \%CV$$

TEa represents the clinically allowable combined error derived from biological variation data[3] or from regulatory standards.[7] Bias captures the systematic component of error measured through EQAS participation, while %CV captures the random component measured through IQC records. A sigma score of 6 or above denotes World Class performance, where the method generates fewer than 3.4 defective results per million opportunities. A score below 3 indicates that the proportion of out-of-specification results is clinically unacceptable, and reporting should be suspended pending corrective action.

A complementary index, the Quality Goal Index (QGI), defined as Bias ÷1.5 %CV, identifies which component of analytical error is dominant.[5] Where QGI falls below 0.8, random error (imprecision) predominates, which typically traces to reagent lot inconsistency, inadequate QC reconstitution, or instrument mechanical issues. When QGI exceeds 1.2, systematic error (inaccuracy) predominates, generally indicating a calibration failure or matrix interference. Values between 0.8 and 1.2 indicate a mixed pattern.[14] This distinction is practically important because the corrective action for each error type differs substantially: imprecision problems require reagent and instrument interventions, while inaccuracy problems require calibration management.

Published sigma studies from India are predominantly single-centre evaluations of one concentration level — usually the normal QC range — at metropolitan teaching hospitals.[6,7] Testing at a single QC level is methodologically limiting because analytical behaviour at pathological concentrations may differ materially from behaviour at normal concentrations, owing to nonlinear assay kinetics, reagent saturation, and concentration-dependent matrix effects.[8,9] A test that appears acceptable at normal QC values may, in fact, perform poorly at pathological concentrations — precisely where results carry the greatest diagnostic weight. Moreover, studies from central Indian laboratories, including the Marathwada region of Maharashtra, are not represented in the published literature, leaving a gap in regional quality benchmarking.

The present study was designed to fill this gap. Its objectives were: (i) to compute Six Sigma scores for 21 routine biochemistry analytes at both normal (Level 1) and pathological (Level 2) IQC concentrations over six months; (ii) to apply appropriate, level-differentiated bias sources for more accurate sigma estimation; (iii) to calculate QGI at both levels to classify the dominant error type and guide corrective action; and (iv) to assign sigma-guided QC protocols to each analyte. This is, to our knowledge, the first report of dual-level sigma metrics with level-specific bias sources from a clinical biochemistry laboratory hailing from Marathwada Region of Maharashtra.

MATERIALS AND METHODS

2.1 Study Setting and Design

This prospective observational study was conducted in the Department of Biochemistry at a tertiary-care teaching hospital in Aurangabad (Chhatrapati Sambhajinagar), Maharashtra, India, between October 2025 and March 2026 (a six-month period). The work was conducted under the quality management framework of ISO 15189:2022.[18]

2.2 Analytical System

All measurements were made on a Siemens Atellica 1900 CI integrated biochemistry analyser using photometric, enzymatic, and turbidimetric reaction principles. Calibrators were manufacturer-supplied and traceable to higher-order reference methods. Calibration was verified when a new reagent lot was opened and after any unplanned instrument maintenance.

2.3 Analytes

Twenty-one analytes were studied: Albumin, Alkaline Phosphatase, ALT, Amylase, AST, Bilirubin Total, Calcium, Chloride, HDL Cholesterol, LDL Cholesterol, Total Cholesterol, Creatinine, Glucose, Iron, Potassium, Total Protein, Sodium, Triglyceride, UIBC, Urea Nitrogen, and Uric Acid. Three analytes (Amylase, Calcium, Uric Acid) had no Level 2 QC material available throughout the study and were therefore assessed only at Level 1. All analytes participated in the external quality scheme for the full study duration.

2.4 Internal Quality Control

Two third-party Bio-Rad lyophilised IQC materials were used: Level 1 (normal range, analyte concentrations within the healthy adult reference interval) and Level 2 (pathological range, concentrations above the upper reference limit). Both were run in duplicate at the start of each analytical session. Monthly %CV values were calculated from the combined within-run and between-run variance of the six-month IQC records.

2.5 Bias Measurement

Bias was assessed using two approaches as described by Thelen et al.[17] For Level 1, the monthly EQAS peer-group bias was computed as: Bias% = [(laboratory mean − assigned value) / assigned value] × 100, and the six-month mean was taken. For Level 2, an IQC-based bias specific to the pathological concentration range was used, calculated from the systematic offset observed in Level 2 IQC records. This approach recognises that EQAS peer-group statistics are anchored to the overall method population and may not accurately represent the systematic error operating at pathological concentrations in a specific analyser-reagent combination.

2.6 Sigma Score Calculation

Sigma scores were computed independently for each analyte at each QC level:

$$\sigma = (\text{TEa} - \text{Bias}\ \%) \div \%\text{CV}$$

TEa values were taken from the Ricos biological variation database (desirable level)[3] or CLIA 1988 regulatory limits,[7] whichever was more stringent for each analyte. Performance was graded: σ ≥ 6 = World Class; 5–6 = Excellent; 4–5 = Good; 3–4 = Marginal; <3 = Unacceptable.

2.7 Quality Goal Index

For each analyte at each level, QGI = Bias% ÷ 1.5%CV. Values below 0.8 indicate imprecision as the dominant error; values above 1.2 indicate inaccuracy; intermediate values indicate mixed error, per the criteria of Goswami et al.[5,14]

2.8 QC Protocol Assignment

QC protocols were assigned to each analyte based on its sigma score, using Westgard Sigma Rules[1,4] and OPSpecs chart guidance. The Level 2 sigma was used for definitive protocol assignment, as pathological concentrations are of greater clinical relevance.

2.9 Ethics

The study used de-identified, aggregated QC and EQAS performance records. No patient samples were obtained for research, and no patient-identifiable data were accessed.

RESULTS

3.1 Overall Sigma Distribution

Table 1 contains the complete sigma data for all 21 analytes at both QC levels. At Level 1 (EQAS bias; normal %CV), scores spanned 2.29 (UIBC) to 15.00 (Amylase). Ten analytes (47.6%) were World Class: Alkaline Phosphatase (12.45), Amylase (15.00), ALT (6.78), Glucose (6.45), HDL Cholesterol (13.45), Iron (9.22), LDL Cholesterol (8.31), Total Protein (6.80), Triglyceride (9.54), and Uric Acid (13.93). Five analytes were Excellent: Albumin (5.99), Bilirubin Total (5.41), Sodium (5.68), Total Cholesterol (5.68), and Creatinine (borderline, 4.20 at L1 rising to 5.47 at L2). Five were Good or Marginal: AST (4.64), Calcium (4.72), Chloride (4.07), Potassium (3.13), and Urea Nitrogen (3.52). UIBC (σ = 2.29) was the sole Unacceptable analyte.

At Level 2 (IQC bias; pathological %CV; 18 analytes with data available), scores ranged from 2.92 (UIBC) to 17.39 (ALP). Eleven of 18 evaluable analytes (61.1%) were World Class. Taken together, roughly 68% of analytes at Level 1 and 89% of evaluable analytes at Level 2 met Excellent or World Class criteria, reflecting broadly satisfactory analytical performance.

Table 1: Six Sigma scores at Level 1 (EQAS bias; normal IQC %CV) and Level 2 (IQC-derived bias; pathological IQC %CV) for 21 routine biochemistry analytes, October 2025–March 2026. TEa = Total Allowable Error. N/A = Level 2 data not available (Amylase, Calcium, Uric Acid)

Analyte	TEa	LEVEL 1 – EQAS Bias/Normal IQC %CV				LEVEL 2 – IQC Bias/Pathological IQC %CV			
		Bias (%)	% CV	σ	Grade	Bias (%)	% CV	σ	Grade
Albumin	10%	1.50	1.67	**5.99**	Excellent	0.49	2.01	**4.73**	Good
Alkaline Phosphatase	30%	3.14	2.41	**12.45**	World Class	0.44	1.70	**17.39**	World Class
ALT	20%	2.61	2.95	**6.78**	World Class	2.03	2.12	**8.48**	World Class

Amylase	30%	8.90	2.00	**15.00**	World Class	N/A	N/A	**N/A**	N/A
AST	20%	0.92	4.31	**4.64**	Good	2.66	3.58	**4.84**	Good
Bilirubin Total	20%	3.46	3.70	**5.41**	Excellent	2.97	2.80	**6.08**	World Class
Calcium	10%	0.90	2.12	**4.72**	Good	N/A	N/A	**N/A**	N/A
Chloride	5%	1.02	1.23	**4.07**	Good	1.47	1.16	**3.04**	Marginal
HDL Cholesterol	30%	1.93	2.23	**13.45**	World Class	2.36	2.97	**9.31**	World Class
LDL Cholesterol	30%	2.38	3.61	**8.31**	World Class	3.16	4.67	**5.75**	Excellent
Total Cholesterol	10%	1.02	1.76	**5.68**	Excellent	0.92	1.87	**4.86**	Good
Creatinine	15%	2.32	3.57	**4.20**	Good	0.78	2.60	**5.47**	Excellent
Glucose	10%	1.60	1.55	**6.45**	World Class	0.90	1.40	**6.50**	World Class
Iron	20%	2.38	2.17	**9.22**	World Class	1.64	2.25	**8.16**	World Class
Potassium	5%	0.94	1.60	**3.13**	Marginal	0.69	1.21	**3.56**	Marginal
Total Protein	10%	1.96	1.47	**6.80**	World Class	0.53	1.57	**6.03**	World Class
Sodium	5%	0.82	0.88	**5.68**	Excellent	0.57	0.82	**5.40**	Excellent
Triglyceride	25%	2.55	2.62	**9.54**	World Class	1.08	4.08	**5.86**	Excellent
UIBC	20%	7.05	8.73	**2.29**	Unacceptable	2.35	6.04	**2.92**	Unacceptable
Urea Nitrogen	9%	0.90	2.56	**3.52**	Marginal	1.42	2.18	**3.40**	Marginal
Uric Acid	17%	1.49	1.22	**13.93**	World Class	N/A	N/A	**N/A**	N/A

3.2 Notable Inter-Level Differences

Several analytes showed meaningful shifts between levels. ALP gained notably from L1 (σ = 12.45) to L2 (σ = 17.39), driven by a much lower IQC-derived bias at the pathological level (0.44% vs. 3.14% EQAS bias). Creatinine moved from Good at L1 (σ = 4.20) to Excellent at L2 (σ = 5.47), again reflecting a lower pathological-level bias (0.78% vs. 2.32%). Bilirubin Total reached World Class at L2 (σ = 6.08) after scoring Excellent at L1 (σ = 5.41). Moving in the opposite direction, Albumin fell from Excellent at L1 (σ = 5.99) to Good at L2 (σ = 4.73), and Chloride dropped from Good at L1 (σ = 4.07) to Marginal at L2 (σ = 3.04), consistent with its narrow TEa of 5% and higher imprecision at elevated concentrations.

3.3 UIBC — the Sole Unacceptable Analyte

UIBC did not reach an acceptable sigma level at either control concentration (L1: σ = 2.29; L2: σ = 2.92). The combination of the highest EQAS bias in the study (7.05%) and markedly elevated %CV at both levels (L1: 8.73%, L2: 6.04%) produced these low scores. The wide month-to-month EQAS bias variability observed throughout the study period points to intermittent analytical instability rather than a fixed systematic offset. Until the root cause is identified and corrected, UIBC results should be reported with an explicit quality caveat and clinicians should be informed of the limitation.

3.4 QGI and Root Cause Classification

Table 2 shows the QGI analysis for all analytes. Imprecision was the leading error type at Level 1 (17 of 21 analytes, QGI < 0.8) and at Level 2 (16 of 18 evaluable analytes, QGI < 0.8). Inaccuracy-dominant patterns were found for Amylase (QGI 2.96). Mixed patterns were recorded for Total Protein (0.88), Uric Acid (0.81), and ALP (0.86) at level 1 and Chloride (0.84), ALT (0.93) at Level 2. The wide prevalence of imprecision-dominant errors across the analyte menu identifies reagent consistency, QC material handling, and instrument maintenance as the priority areas for quality improvement in this laboratory.

Table 2: Quality Goal Index (QGI), dominant error classification, assigned QC protocol, and corrective action guidance at Level 1 and Level 2 for all 21 analytes. QGI <0.8: imprecision dominant; QGI 0.8–1.2: mixed; QGI >1.2: inaccuracy dominant. QC protocol assignment based on sigma tier per Westgard Sigma Rules. N/A = Level 2 data unavailable

Analyte	Bias (%)	QGI L1	Error Type L1	QGI L2	Error Type L2	QC Protocol	Corrective Action
Albumin	1.50	**0.59**	*Imprecision*	**0.16**	*Imprecision*	1_{3s} +2_{2s} ; N=2 / Westgard MR; N=4	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Alkaline Phosphatase	3.14	**0.86**	*Mixed*	**0.17**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
ALT	2.61	**0.59**	*Imprecision*	**0.93**	*Mixed*	1_{3s} ; N=2 / 1_{3s} ; N=2	Consideration of integrated approach-assessment of reagent shelf-life , recalibration , evaluation of carryover & screening the samples for Lipemia & haemolysis
Amylase	8.90	**2.96**	*Inaccuracy*	**N/A**	*N/A*	1_{3s} ; N=2 / —	Verification of calibration ,confirmation of calibrator traceability, assessment of matrix interference & monitoring of EQAS peer group trend

AST	0.92	**0.14**	*Imprecision*	**0.49**	*Imprecision*	Westgard MR; N=4 / Westgard MR; N=4	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Bilirubin Total	3.46	**0.62**	*Imprecision*	**0.70**	*Imprecision*	1_{3s} +2_{2s} ; N=2 / 1_{3s} ; N=2	Consideration of integrated approach-assessment of reagent shelf-life , recalibration , evaluation of carryover & screening the samples for Lipemia & haemolysis
Calcium	0.90	**0.28**	*Imprecision*	**N/A**	*N/A*	Westgard MR; N=4 / —	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Chloride	1.02	**0.55**	*Imprecision*	**0.84**	*Mixed*	Westgard MR; N=4 / Multirule; N=6	Verification of calibration ,confirmation of calibrator traceability, assessment of matrix interference & monitoring of EQAS peer group trend
HDL Cholesterol	1.93	**0.57**	*Imprecision*	**0.52**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
LDL Cholesterol	2.38	**0.43**	*Imprecision*	**0.45**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} +2_{2s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Total Cholesterol	1.02	**0.38**	*Imprecision*	**0.32**	*Imprecision*	1_{3s} +2_{2s} ; N=2 / Westgard MR; N=4	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Creatinine	2.32	**0.43**	*Imprecision*	**0.23**	*Imprecision*	Westgard MR; N=4 / 1_{3s} +2_{2s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Glucose	1.60	**0.68**	*Imprecision*	**0.76**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Iron	2.38	**0.73**	*Imprecision*	**0.48**	*Imprecision*	1_{3s} ; N=2 1_{3s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Potassium	0.94	**0.39**	*Imprecision*	**0.38**	*Imprecision*	Multirule; N=6 / Multirule; N=6	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Total Protein	1.96	**0.88**	*Mixed*	**0.22**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Sodium	0.82	**0.62**	*Imprecision*	**0.46**	*Imprecision*	1_{3s} +2_{2s} ; N=2 / 1_{3s} +2_{2s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Triglyceride	2.55	**0.64**	*Imprecision*	**0.17**	*Imprecision*	1_{3s} ; N=2 / 1_{3s} +2_{2s} ; N=2	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
UIBC	7.05	**0.42**	*Imprecision*	**0.25**	*Imprecision*	Cease—Investigate / Cease—Investigate	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Urea Nitrogen	0.90	**0.19**	*Imprecision*	**0.43**	*Imprecision*	Multirule; N=6 / Multirule; N=6	Evaluation of lot stability, checking QC reconstitution, pipette calibration & equipment PM records
Uric Acid	1.49	**0.81**	*Mixed*	**N/A**	*N/A*	1_{3s} ; N=2 / —	Verify calibration; confirm calibrator traceability; assess matrix interference; monitor EQAS peer-group trend.

DISCUSSION

This study reports the analytical quality profile of a routine clinical biochemistry laboratory in a tertiary care teaching institute, assessed over six months using dual-level IQC data and level-specific bias sources. The finding that approximately 68% of analytes at Level 1 and 89% at Level 2 reached Excellent or World Class performance is favourable in the context of comparable Indian studies. Panda et al. reported from AIIMS Bhubaneswar that only about half of 19 analytes met the $\sigma \geq 4$ threshold at the normal control level when tested on the Beckman Coulter AU5800,[8] while Gadde and Hm, working at a NABL-accredited laboratory in Bengaluru with the Roche Cobas 6000, found that 10 of 21 analytes achieved World Class performance,[9] a proportion similar to our own Level 1 result. The higher proportion at Level 2 in our laboratory is explained by improved analytical precision at higher analyte concentrations — a physicochemical consequence of

improved signal-to-noise ratios in photometric and enzymatic assay systems.[3,4]

The exceptionally high sigma values for Alkaline Phosphatase (σ = 12.45 at L1, 17.39 at L2) and Amylase (σ = 15.00 at L1) are well supported by published data. Garg et al., in a New Delhi study on the Beckman Coulter AU 680, identified Amylase as the only analyte achieving World Class performance at both QC levels, attributing this to the stability of the kinetic p-nitrophenyl substrate reaction and high substrate concentrations in clinical specimens.[10] Peng et al. similarly found ALP, Amylase, Creatinine, Uric Acid, and Sodium consistently World Class at Level 2 across multiple platforms.[12] The marked improvement in ALP sigma from Level 1 to Level 2 in our dataset is a direct consequence of applying IQC-derived bias at the pathological level (0.44%) rather than the overall EQAS bias (3.14%), validating the dual-bias approach proposed by Thelen et al.[17]

UIBC warrants specific discussion. Ercan et al., reporting on 27 analytes with the Beckman Coulter AU5800, noted that UIBC showed a combined imprecision and inaccuracy signature on QGI analysis, with most other analytes performing satisfactorily.[14] The difficulty with UIBC measurement is inherent to the methodology: the indirect FerroZine colorimetric technique saturates available transferrin iron-binding sites with excess ferrous iron and then quantifies the residual unbound fraction — an inverse measurement susceptible to interference from haemolysis, lipaemia, and protein matrix variability between concentration levels.[11] The imprecision-dominant QGI at Level 2 in our study (0.25) specifically points to reagent instability at elevated iron-binding capacity concentrations. A systematic response should address reagent lot verification before clinical use; pre-analytical sample quality screening; QC material reconstitution practices; and evaluation of direct TIBC measurement as a technically superior alternative.

The QGI findings carry a practical message that extends beyond individual analytes. The preponderance of imprecision-dominant errors across the menu implies that the laboratory's highest-priority quality investment is not recalibration but consistent reagent management. Goswami et al., in their foundational paper establishing the QGI framework, showed that imprecision-classified problems in routine chemistry reliably trace to reagent reconstitution conditions, storage temperature, and instrument mechanical maintenance rather than to calibration drift.[5] Panda et al. reached a concordant conclusion from their Indian tertiary care data, identifying QC reconstitution errors, incorrect storage, and air bubble introduction as the dominant root causes in imprecision-dominant analytes.[8] For the minority of analytes in our study where inaccuracy dominates — specifically Amylase at level 1 — fresh calibration, calibrator lot traceability review, and EQAS trend monitoring are the appropriate corrective steps.

Four analytes in the Marginal or Good range merit individual consideration. Potassium (σ = 3.13 at L1, 3.56 at L2) and Chloride (σ = 4.07 at L1, 3.04 at L2) carry the tightest TEa of any analyte in this study — 5% each — leaving almost no margin for error. Chernet and Tamiru, evaluating a clinical chemistry reference laboratory in Ethiopia with Cobas 6000, encountered similar difficulty with Chloride, attributing the poor sigma to the stringent CLIA electrolyte tolerance.[13] The decline in Chloride sigma between levels in our data suggests that imprecision worsens at elevated chloride concentrations, an observation that should prompt investigation of the photometric or ion-selective electrode response across the working range. Creatinine's improvement from Good at L1 to Excellent at L2 illustrates the bias methodology effect: the higher L1 EQAS bias (2.32%) reflects inter-method variability across the EQAS peer group rather than the instrument's true systematic error at clinical decision concentrations, as discussed by Thelen et al.[17]

The dual-level design itself generated clinically relevant information that a single-level evaluation would have missed. Calcium, for example, scored Good at Level 1 (σ = 4.72) but had no Level 2 data, leaving its performance in the hypercalcaemia range entirely unknown. Both Panda et al. and Gadde and Hm documented significant inter-level sigma differences for multiple analytes in their studies,[8,9] reinforcing the value of routine dual-level assessment. The 2025 IFCC recommendations on IQC practice, issued in alignment with ISO 15189:2022, endorse sigma-guided, risk-proportionate QC frequency and explicitly support multi-level assessment for comprehensive analytical monitoring.[16]

The QC rationalisation implication of these findings is operationally significant. Applying uniform intensive multirule protocols to all 21 analytes — as is common in many Indian laboratories — consumes reagents, technician time, and review capacity without proportional benefit for well-performing tests. Phansalkar et al. demonstrated that sigma-guided QC rule selection reduces false rejections without compromising error detection.[15] Van der Laan's implementation of TEa and Six Sigma-based IQC design at a Dutch hospital produced measurable savings in reagent expenditure, fewer recalibrations triggered by false alarms, and improved workflow throughput.[20] Concentrating enhanced monitoring on the tests that genuinely require it — primarily UIBC and, to a lesser extent, Potassium, Chloride, and Urea Nitrogen — while applying streamlined monitoring to the 10–11 World Class analytes would produce meaningful efficiency gains without sacrificing quality.

Several limitations must be noted. The study was conducted within a single laboratory using one analyser, providing a controlled and internally consistent assessment, though broader multicentre evaluations would be valuable to further establish

generalisability across diverse laboratory settings. TEa targets from the Ricos database, while widely used, are based on European populations and may not fully reflect Indian biological variation, underscoring the need for regional data. The six-month period captures short- to medium-term performance, with longer studies needed for extended trends.

CONCLUSION

This six-month dual-level sigma metric evaluation of 21 routine biochemistry analytes demonstrates that overall analytical performance in a tertiary care teaching laboratory is robust, with most parameters achieving Excellent to World Class quality in line with established benchmarks. UIBC was the only analyte to show consistently suboptimal performance across both concentration levels, highlighting the need for focused corrective intervention. Imprecision emerged as the principal contributor to analytical error, indicating that targeted improvements in reagent management, quality control practices, and instrument maintenance are likely to yield the greatest benefit. Importantly, dual-level assessment revealed clinically relevant, concentration-dependent variations in performance that would not have been detected using single-level evaluation. These findings support the routine adoption of dual-level sigma monitoring and reinforce the utility of sigma-stratified quality control strategies, enabling optimisation of laboratory resources while maintaining high standards of analytical reliability.

Conflict of Interest

The authors declare no conflict of interest. No external financial support was received. EQAS data used in this study were generated through the laboratory's routine participation in its established quality scheme; no samples were procured specifically for this research.

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