Original Article

Cukurova Anestezi ve Cerrahi Bilimler Dergisi

Analytical Performance Evaluation of Routine Biochemical Tests Using Sigma Metrics and Quality Goal Index According to CLIA 2025 Targets

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Abstract

Background: Accurate analytical results are essential for clinical decision-making. The revised CLIA 2025 criteria have introduced stricter total allowable error (TEa) limits, requiring reassessment of laboratory analytical performance using advanced quality tools. **Methods:** In this retrospective observational study, internal quality control (IQC) and external quality assessment (EQA) data from 2023 were analyzed. Imprecision was evaluated using coefficient of variation (CV%), and accuracy was assessed using bias (%). Sigma values were calculated according to updated CLIA 2025 TEa limits. Quality Goal Index (QGI) analysis was performed for tests with low sigma performance. **Results:** Considerable variability was observed among analytes. AST demonstrated excellent sigma performance (≥6), whereas HDL showed good analytical performance within the 3–6 sigma range. Chloride, BUN, and triglycerides yielded sigma values <3. QGI analysis indicated that reduced performance was mainly attributable to imprecision. **Conclusion:** The combined use of sigma metrics and QGI provides an effective framework for evaluating laboratory analytical performance under CLIA 2025 criteria and supports risk-based quality improvement strategies.

Keywords: Sigma Metrics; Total Analytical Error; Quality Goal Index; CLIA 2025; Clinical Biochemistry

1. Introduction

Producing accurate and reliable results in clinical laboratories is of critical importance for appropriate diagnosis and effective treatment management. Internal quality control (IQC) and external quality assessment (EQA) practices are standard approaches used to monitor analytical performance[1,2].

IQC procedures allow the assessment of test repeatability through routine daily analysis of control materials, thereby monitoring random error (imprecision). External quality assessment programs enable interlaboratory comparisons, revealing deviations from target values, thus primarily evaluating systematic error (bias)[2,3].

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Total Analytical Error (TAE) represents the combined effect of all sources of error—random and systematic—affecting an analytical result[4]. Total allowable error (TEa) defines the maximum clinically acceptable limit of total error for a given analyte[5].

The CLIA regulations were updated in 2024, and with the revised criteria that came into effect in 2025, allowable error limits for many biochemical tests have been further tightened[4]. For example, the TEa limit for alkaline phosphatase (ALP) was reduced from ±30% to ±20%; for alanine aminotransferase (ALT), from ±20% to ±15%; and for albumin, from ±10% to ±8% under CLIA 2025 criteria[4].

The Six Sigma methodology, originally adapted from industry, measures process capability by expressing defects per million opportunities (DPMO). The sigma metric is calculated using the formula: Sigma = (TEa − Bias%) / CV%, providing a numerical representation of analytical quality level[2]. Tests with sigma values <3 are classified as unacceptable, while a sigma level of 6 is regarded as "world-class" performance[5].

The Quality Goal Index (QGI) is a complementary parameter that helps identify the underlying source of poor test performance. QGI is defined as QGI = Bias% / (1.5 × CV%). A QGI value <0.8 indicates imprecision-dominant performance deficiency, while QGI >1.2 suggests bias as the predominant source of error[5].

Despite the increasing use of sigma metrics in laboratory quality assessment, there is limited data evaluating routine biochemical tests under the revised CLIA 2025 framework. Therefore, this study aims to address this gap by providing a comprehensive assessment of analytical performance using sigma metrics and QGI based on updated CLIA targets.

2. Aim

The aim of this study was to comprehensively evaluate the analytical performance of routine biochemical tests performed on autoanalyzer systems in a tertiary care medical biochemistry laboratory using quality management tools. Sigma metric values were determined for each test based on TEa, bias, and CV%, and analytical performance was evaluated accordingly. Furthermore, for tests with low sigma metric values, QGI analyses were conducted to identify whether reduced performance was primarily attributable to imprecision or inaccuracy (bias), thereby providing insight into laboratory quality improvement efforts.

3. Materials and Methods

3.1. Study Design and Setting

This study was conducted as a retrospective observational investigation in a tertiary care medical biochemistry laboratory. Ethics committee approval was obtained (decision dated 08 Oct 2025) and the study was conducted in accordance with the principles of the Declaration of Helsinki. Internal and external quality control data of routine biochemical tests performed in 2023 were analyzed.

The evaluated test parameters included albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), chloride (Cl), total cholesterol (CHOL), creatinine (CREA), glucose (GLU), high-density lipoprotein cholesterol (HDL), lactate dehydrogenase (LDH), total protein (TP), triglycerides (TG), and blood urea nitrogen (BUN).

3.2. Data Collection

IQC data were obtained from daily quality control results recorded between 1 January 2023 and 1 January 2024. Two levels of control materials (low and high concentration) were analyzed daily. Control results rejected due to noncompliance with Westgard rules were excluded. Monthly CV% values were calculated using the formula CV% = (SD / mean) × 100. A composite CV was derived as: $CV_{total}\% = \sqrt{[(CV_1\%^2 + CV_2\%^2) / 2]}$.

3.3. External Quality Assessment (EQA) Data

EQA data were obtained from a monthly external quality assessment program throughout 2023. Percentage bias values were calculated as: Bias% = [(Laboratory result − EQA target value) / EQA target value] × 100[5]. A total of 12 EQA samples corresponding to 2023 were evaluated and annual mean Bias% values were recorded.

3.4. Sigma and QGI Calculations

Sigma metric was calculated as: σ = (TEa% − Bias%) / CV%, using updated CLIA 2025 TEa limits[8]. Sigma values were also calculated on a monthly basis. Tests with sigma values approaching 6 were considered excellent; values below 3 were regarded as insufficient; and 3–6 sigma indicated moderate performance with potential for improvement[1].

QGI was calculated for analytes with sigma values below 4 as: QGI = Bias% / (1.5 × CV%). QGI <0.8 indicated imprecision-dominant deficiency; QGI >1.2 indicated bias-dominant deficiency; and QGI 0.8–1.2 indicated mixed contribution[5].

3.5. Statistical Analysis

All data calculations and analyses were performed using SPSS version 26.0. Sigma and QGI values were summarized using tables and graphical representations. Normality of continuous variables was assessed descriptively; as the study was methodological in nature, no inferential statistical comparisons were performed. Performance classification was conducted according to the internationally accepted Westgard approach.

4. Results

4.1. Sigma Metric Performance of Biochemical Tests

Sigma metric values calculated for the thirteen biochemical analytes based on 2023 IQC and EQA data are presented in Table 1. Considerable variability was observed across analytes. AST demonstrated the highest sigma value (6.37), corresponding to excellent ("world-class") analytical performance. HDL also showed favorable performance, with a sigma value of 5.10, placing it within the upper range of the moderate category. The majority of tests — ALB (3.18), ALT (4.16), ALP (3.31), CHOL (3.97), CREA (3.19), GLU (3.91), LDH (3.55), and TP (3.85) — fell within the moderate performance range (3–6 sigma), indicating acceptable but improvable analytical quality. In contrast, three analytes yielded sigma values below 3, reflecting low (unacceptable) performance: chloride (2.46), triglycerides (2.86), and BUN (2.90). Chloride demonstrated the lowest sigma value among all tests evaluated.

Table 1. Sigma metric results of the analyzed biochemical tests based on mean annual values (2023)

Analyte	Mean Sigma Value	Performance Category
ALB	3.18	Moderate
ALT	4.16	Moderate
ALP	3.31	Moderate
AST	6.37	Excellent
Cl	2.46	Low
CHOL	3.97	Moderate
CREA	3.19	Moderate
GLU	3.91	Moderate
HDL	5.10	Moderate
LDH	3.55	Moderate
TP	3.85	Moderate
TG	2.86	Low
BUN	2.90	Low

Performance categories were interpreted according to Westgard sigma criteria (<3: low; 3–6: moderate; ≥6: excellent). Performance categories were interpreted according to Westgard sigma criteria (<3: low; 3–6: moderate; ≥6: excellent). ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cl, chloride; CHOL, total cholesterol; CREA, creatinine; GLU, glucose; HDL, high-density lipoprotein cholesterol; LDH, lactate dehydrogenase; TG, triglycerides; TP, total protein.

4.2. Analyzer-Based Distribution of Low-Sigma Error Sources

For analytes with low sigma performance, error sources were further examined across the five autoanalyzers, as shown in Table 2. Across all instruments, CV-related (imprecision-dominant) errors were markedly more frequent than bias-related errors. Analyzer 5 exhibited the highest proportion of CV-related errors (47.1%), followed by Analyzer 3 (44.2%) and Analyzer 1 (37.5%), while Analyzer 4 showed the lowest proportion (35.6%). Bias-related errors were comparatively infrequent across all analyzers, ranging from 1.9% (Analyzer 4) to 7.7% (Analyzer 1). Combined CV and bias errors were most prominent in Analyzer 2 (10.6%), whereas the remaining analyzers showed combined error rates below 7%. Overall, imprecision emerged as the predominant error source across all five instruments.

Table 2. Analyzer-based distribution of low-sigma error sources

Autoanalyzer	CV-Related Errors n (%)	Bias-Related Errors n (%)	Combined Errors n (%)
Analyzer 1	39 (37.5)	8 (7.7)	7 (6.7)
Analyzer 2	34 (32.7)	5 (4.8)	11 (10.6)
Analyzer 3	46 (44.2)	4 (3.9)	5 (4.8)
Analyzer 4	37 (35.6)	2 (1.9)	3 (2.9)
Analyzer 5	49 (47.1)	3 (2.9)	4 (3.8)

CV-related errors indicate imprecision-dominant performance deficiencies as identified by QGI analysis.

4.3. QGI-Based Distribution of Analytical Error Sources

QGI analysis performed for analytes with sigma values below 4 is summarized in Table 3. Of the total error sources identified, 39.4% were attributable to imprecision (CV-related, QGI <0.8), while only 4.2% were attributable to bias (QGI >1.2). A smaller proportion, 5.8%, reflected a combined contribution of both CV and bias (QGI 0.8–1.2). These findings indicate that imprecision, rather than systematic bias, was the predominant source of reduced analytical performance among the low-sigma tests evaluated in this study.

Table 3. Distribution of analytical error sources based on QGI analysis

Error Source	Proportion (%)	Interpretation
Imprecision (CV)	39.4	Predominant random error
Bias	4.2	Accuracy-related issue
Combined CV and Bias	5.8	Mixed error source

QGI <0.8 indicates imprecision-related errors; QGI >1.2 indicates bias-related errors; 0.8–1.2 suggests combined contribution.

5. Discussion

5.1. Distribution of Sigma Metrics

Evaluation of sigma metric performance distribution revealed marked differences in quality levels among the assays. Sigma values greater than 6 are commonly described as "world-class" performance, whereas sigma values below 3 indicate poor or unacceptable performance[6]. This distribution is consistent with previous studies; for instance, one study reported that 46% of 60 tests exhibited sigma values below 6, with as many as 20% falling below 3 sigma[7].

Tests with high sigma values (e.g., AST) do not require unnecessarily frequent IQC procedures, while low-sigma tests necessitate more frequent monitoring. For tests with sigma values <3, multiple QC rules (e.g., 1_3s, 2_2s, and R_4s) should be applied simultaneously and the frequency of daily QC measurements should be increased[6].

5.2. Instrument-Based Comparisons

Although all instruments used in the present study were of the same brand and model, measurable inter-instrument performance differences were identified. These discrepancies may be associated with subtle calibration shifts, different reagent lots, or maintenance performed by different operators. Previous studies have emphasized that instrument-related performance variability may be influenced by analytical technology, calibration procedures, and instrument maintenance status[6].

QGI analyses performed for each instrument revealed that performance deficiencies were predominantly driven by random error (CV-related imprecision), although bias-related differences were occasionally observed between instruments. To minimize inter-instrument variability, strict adherence to standardized calibration protocols across all analyzers and synchronized routine maintenance are of critical importance.

5.3. Test-Based Analysis

Test-based analysis indicated that chloride (Cl), blood urea nitrogen (BUN), and triglyceride (TG) assays yielded sigma values below 3. This finding may be partly attributable to the relatively stringent TEa limits defined for these analytes under CLIA 2025: the TEa for chloride is ±5%, while relatively low allowable error limits are specified for BUN (±9%). Previous studies have similarly reported that electrolytes and nitrogenous metabolites frequently exhibit lower sigma performance[6].

In contrast, AST demonstrated sigma performance at or above the Six Sigma level, and HDL exhibited good performance within the 3–6 sigma range. An international multicenter study reported that approximately 69% of 115 tests demonstrated sigma values ≥6, with enzyme assays such as ALP, ALT, and AST demonstrating sigma values exceeding 6[10].

5.4. QGI-Based Analysis of Error Sources

QGI analysis indicated that reduced analytical performance was predominantly driven by random errors associated with imprecision. When all instruments and tests were evaluated collectively, 39.4% of the identified error sources were attributed to imprecision (CV-related issues), while only 4.2% were attributable to bias. This finding is consistent with the literature; in a multicenter study by Wauthier et al., imprecision was identified as the primary source of poor performance in 52% of low-performing tests in one laboratory[7].

The predominance of CV-related issues indicates a need to focus on measures aimed at reducing analytical variability, including increasing the frequency of instrument maintenance and calibration, implementing more frequent IQC procedures for critical assays, and reinforcing operator training.

5.5. Overall Evaluation

The CLIA quality goals, revised in 2024 and implemented as of 2025, have further tightened TEa limits for many tests. For example, TEa targets were updated to ±15% for ALT (from ±20%), ±8% for albumin (from ±10%), and ±20% for ALP (from ±30%) under CLIA 2025 criteria[9]. All calculations were performed using these updated, more stringent targets. The findings indicate that many assays were able to meet these demanding quality goals, while BUN, triglycerides, and chloride showed inadequate performance with sigma values consistently below 3.

The CLIA amendments that came into effect in July 2024 constitute the most substantial revision since 1992, mandating laboratories to update and optimize their analytical quality practices[8]. For tests with low sigma values, existing QC procedures may be insufficient, and implementation of multirule strategies (e.g., replacing 1_2s with 1_3s rules in combination) may be warranted[6].

This study has several limitations. First, its retrospective design may limit control over pre-analytical and analytical variables. Second, CV and bias values were calculated from routine IQC and EQA data, which may be affected by reagent lot variability, calibration differences, and instrument-related factors. Third, the use of monthly aggregated CV values may mask short-term analytical fluctuations. Finally, as this was a single-center study, generalizability may be limited.

6. Conclusion

In-depth analysis of the study findings objectively delineated analytical performance relative to the CLIA 2025 quality targets and provided direction for future quality improvement initiatives. The sigma metric approach and QGI analysis proved to be valuable tools for continuous monitoring and enhancement of laboratory performance. For tests demonstrating high sigma values, existing QC procedures appear adequate and QC frequency may be optimized. For low-sigma analytes, targeted corrective actions—including instrument maintenance, calibration optimization, and operator training—should be prioritized. Future efforts should focus on updating internal quality control procedures using a risk-based, analyte-specific approach aligned with the updated CLIA 2025 targets.

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Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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