

Analytical performance evaluation of biochemical parameters of a public hospital laboratory

Avaliação do desempenho analítico de parâmetros bioquímicos do laboratório de análises clínicas de um hospital público

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ABSTRACT

Introduction: Every process is subject to variation in the results of repeated analyses, that is why quality control was conceived to measure and evaluate this variation. In addition to the daily analysis of control results, a long-term assessment should evaluate the analytical performance of each method for inaccuracy and imprecision according to quality specifications (QS). **Objective:** This study aimed to evaluate the analytical performance of biochemical parameters of the Clinical Analysis Laboratory (CAL) of a Brazilian university hospital, according to the QS based on biological variation and comparing to an international laboratory. **Material and method:** Twenty-six biochemical parameters were analyzed. The QS for coefficient of variation (CV), bias and total error (TE) were calculated according to the intraindividual (CVbi) and interindividual (CVbg) biological variation of the analytes described in the literature. The sigma metric (σ) was also calculated based on desirable TE and Clinical Laboratory Improvement Amendments (CLIA). **Results:** Calcium and sodium analytes were out of the QS for all criteria used (CV, bias and TE) in both laboratories. CAL showed 19% of analytes (albumin, calcium, magnesium, total protein, and sodium) outside the QS in all criteria, and the international laboratory 8%. Using the sigma metric, the analytical performance of CAL was also inferior regarding to the comparative laboratory: 54% of analytes with $\sigma < 3$ versus 38%. **Conclusion:** The sigma metric was more rigorous in the evaluation of the analytical performance of biochemical parameters compared to the QS based on biological variation. The non-achievement of quality goals was not laboratory-specific.

Key words: total quality management; analytical quality control; quality control; benchmarking.

RESUMO

Introdução: Todo processo é passível de variação nos resultados de análises repetidas, por isso, o controle da qualidade foi pensado como forma de mensurar essa variação. Além da análise diária dos resultados de controle, deve-se avaliar, em longo prazo, o desempenho analítico de cada método quanto à imprecisão e à inexatidão de acordo com as especificações da qualidade (EDQ). **Objetivos:** Os objetivos deste estudo foram avaliar o desempenho analítico de parâmetros bioquímicos do Laboratório de Análises Clínicas (LAC) de um hospital universitário brasileiro em relação às EDQ baseadas na variação biológica e compará-lo com o de um laboratório de grande porte internacional. **Material e método:** Foram analisados 26 parâmetros bioquímicos. As EDQ para coeficiente de variação (CV), bias e erro total (ET) foram calculadas de acordo com a variação biológica intraindividual (CVbi) e interindividual (CVbg) dos analitos descritas na literatura. A métrica sigma (σ) também foi calculada com base no ET desejável e no Clinical Laboratory Improvement Amendments (CLIA). **Resultados:** Os analitos cálcio e sódio ficaram fora das EDQ para todos os critérios utilizados (CV, bias e ET) em ambos os laboratórios. O LAC apresentou 19% dos analitos (albumina, cálcio, magnésio, proteínas totais e sódio) fora das EDQ em todos os critérios, e o laboratório de grande porte, 8%. Utilizando a métrica sigma, o desempenho analítico do LAC também foi inferior ao do laboratório comparado: 54% dos analitos com σ

< 3 versus 38%. **Conclusão:** A métrica sigma mostrou-se mais rigorosa na avaliação do desempenho analítico de parâmetros bioquímicos quando em comparação com as EDQ baseadas na variação biológica. O não alcance das metas de qualidade não se mostrou laboratório-específico.

Unitermos: gestão da qualidade total; controle analítico de qualidade; controle de qualidade; benchmarking.

RESUMEN

Introducción: Todo proceso está sujeto a variación en los resultados de los análisis repetidos, por lo que se pensó el control de calidad como una forma de medir esta variación. Además del análisis diario de los resultados de control, debe evaluarse el rendimiento analítico a largo plazo de cada método en cuanto a imprecisiones e inexactitudes según las especificaciones de calidad (EDC). **Objetivos:** Los objetivos de este estudio fueron evaluar el desempeño analítico de los parámetros bioquímicos del Laboratorio de Análisis Clínicos (LAC) de un hospital universitario brasileño en relación con las EDC basadas en la variación biológica y compararlo con el de un gran laboratorio internacional. **Material y método:** Se analizaron 26 parámetros bioquímicos. Las EDC para el coeficiente de variación (CV), el sesgo y el error total (ET) se calcularon según la variación biológica intraindividual (CV_{bi}) e interindividual (CV_{bg}) de los analitos descritos en la literatura. La métrica sigma (σ) también se calculó en base al ET deseable y al Clinical Laboratory Improvement Amendments [(CLIA) – Enmiendas de mejora del laboratorio clínico]. **Resultados:** Los analitos de calcio y sodio estuvieron fuera de las EDC para todos los criterios utilizados (CV, sesgo y ET) en ambos laboratorios. El LAC tenía el 19% de los analitos (albúmina, calcio, magnesio, proteína total y sodio) fuera de las EDC en todos los criterios, y el gran laboratorio, el 8%. Utilizando la métrica sigma, el rendimiento analítico del LAC también fue inferior al del laboratorio de comparación: 54% de analitos con $\sigma < 3$ versus 38%. **Conclusión:** La métrica sigma demostró ser más rigurosa en la evaluación del desempeño analítico de los parámetros bioquímicos en comparación con las EDC basadas en la variación biológica. El incumplimiento de los objetivos de calidad no fue laboratorio-específico.

Palabras clave: gestión de la calidad total; control analítico de calidad; control de calidad; benchmarking.

INTRODUCTION

Every process is subject to variation in the results of repeated analyses, that is why quality control was designed as a way to measure and assess this variation, in addition to indicating opportunities for improvement, in order to keep the results within reliable limits⁽¹⁾.

Through the analytical coefficient of variation (CV), the Internal Quality Control (IQC) assesses the accuracy of an analytical method, that is, how close to each other are the results of the same sample analyzed at different times. The External Quality Control (EQC) evaluates the accuracy of the method through the bias (error), which indicates the proximity of measurement results to the true value. The two parameters are essential in choosing and evaluating the analytical performance of a method and are consistent with the quality of patient results, consequently, in the medical decision-making⁽²⁾.

The accuracy of the analytical method mainly influences the analysis of tests performed for disease monitoring, that is,

the variation of the method should not be greater than the biological variation of the analyte in the individual, which can make it difficult to evaluate the ongoing treatment⁽³⁾. The accuracy of the analytical method, however, is especially related to the diagnosis of any health problem, as the result obtained in the analysis needs to be very close to the accuracy, in order to assess the patient's health condition. However, most laboratory tests are used for both diagnosis and monitoring of diseases, being equally important the precision and accuracy of the method⁽⁴⁾.

In addition to analyzing the IQC and EQC results, it must be evaluated for imprecision and inaccuracy the long-term analytical performance of each method, in accordance with the quality specifications (targets)^(5,6). There are hierarchical models for establishing quality goals, also called analytical quality specifications for CV, bias, and total error (TE) of each laboratory's analytical method⁽⁷⁾.

Keeping the same hierarchical order, after 15 years, the analytical quality specifications were revised and summarized

from five to just three categories: specifications based on clinical decisions, biological variation, and state of the art. In addition, were listed criteria for the analysis and use of biological variation studies^(8,9).

The specification of quality based on biological variation is the most widely used. The method is more advantageous because it is accessible to any laboratory, regardless of size, location, and type, being consolidated with simple models applicable to medical needs⁽⁵⁾. The coefficients of biological variation intra-individual (CVbi) and inter-individual or inter-group (CVbg) are used to define the quality specification (QS) of each parameter under optimal, desirable, and minimum conditions^(3,10).

The Westgard database compiles hundreds of studies and specifies the CVbi and CVbg of most laboratory parameters. However, a recent study, The European Biological Variation Study (EuBIVAS) of the European Federation of Clinical Chemistry and Laboratory Medicine has established values for CVbi and CVbg different from those previously used for some parameters^(11,12).

Since the standardization of results across laboratories around the world, regardless of the analytical platform used, is aimed at improving clinical decisions and patient safety, the analysis and the comparison of the analytical performance represent an important initiative not only in verifying the quality of a laboratory, but providing data on how is going the standardization process among laboratories⁽¹³⁾.

Another way of evaluating and comparing analytical performance is through the Six Sigma methodology, which correlates its metric with the probability of errors (failures) that occurs in a million opportunities, seeking to reduce laboratory costs and increase customer satisfaction. In the Six Sigma model of total quality management, the sigma metric is the central objective measure of quality and the basis for developing quality control. The performance of a process can be measured by the equation adapted for laboratory medicine, where sigma (σ) = (desirable TE – bias%)/CV%. The higher the sigma, the better the performance⁽¹⁴⁻¹⁶⁾.

Increasing quality in the laboratory aims to reduce waste from repeated tests, new collections, wrong diagnoses, and unnecessary treatments. It is difficult to estimate in numbers the total of expenses that can be saved, but it is guaranteed the impact of producing a result with acceptable analytical performance on clinical decisions and patient safety. The objectives of this study are to evaluate the analytical performance of biochemical parameters of a laboratory of a university hospital, in relation to quality specifications based on biological variation and compare it with data from the literature and from another large laboratory.

MATERIAL AND METHOD

The clinical analysis laboratory (CAL) of the Polydoro Ernani de São Thiago University Hospital (HU/UFSC), which has about 200 active beds, is a reference health establishment that, in 2017, treated 444,897 patients, being 127,096 inpatients and 317,801 outpatients care. The HU/UFSC cares for several medical specialties, providing the necessary support for the diagnosis and monitoring of the most varied diseases. In 2017, the CAL performed 692,875 tests, with an average of nearly 10,000 tests per employee. The most analyzed tests in the biochemistry sector were creatinine (41,912), urea (29,623), potassium (25,658), sodium (24,400) and glucose (23,301).

The analytical performance of biochemical parameters was evaluated: lactic acid, uric acid, alanine aminotransferase (ALT), albumin, amylase, aspartate aminotransferase (AST), direct bilirubin (DB), total bilirubin (TB), calcium (Ca), creatine phosphokinase (total CPK), total cholesterol (TC), HDL cholesterol, creatinine, iron (Fe), alkaline phosphatase (ALP), phosphorus (P), gamma-glutamyltransferase (GGT), glucose, lactate dehydrogenase (LDH), lipase, magnesium (Mg), potassium (K), total protein (TP), sodium (Na), triglycerides (TGL) and urea. The respective analytical methods are shown in **Table 1**.

The analytical quality specifications were defined according to the Spanish Society of Clinical Biochemistry (SEQC)⁽¹⁰⁾, using the CVbi and CVbg from the Westgard or EuBIVAS database, shown in Table 1.

The data collection for IQC was performed in the Dimension[®] RxL Max 1 auto analyzer (Siemens, USA) in the Biochemistry Sector of the CAL at the HU/UFSC, which performs the IQC only once a day in the early morning. The analytical performance of the analytes from the university hospital was compared with the respective analytes from the laboratory of the Institute of Clinical Chemistry and Biochemistry [Klinični Inštitut Za Klinično Kemijo in Biokemijo (KIKKB)] Ljubljana University Medical Center (Univerzitetni Klinični Center Ljubljana) Slovenia, which performs the IQC five times a day and uses the Advia[®] 1800 equipment, Siemens (USA) and received ISO 15189 accreditation. The Medical Center has about two thousand beds. In 2017, 6,510,455 exams were carried out, around 46 thousand tests per employee. Data were collected in 2018.

The CV was accessed by analyzing the results from the IQC on the autoanalyzer itself for the two levels of control serum. Were analyzed the control results obtained from January to May 2019, and were considered acceptable the results observed in 95% of the normal distribution, that is, within ± 2 standard deviations (SD) of the mean indicated by the control manufacturer.

TABLE 1 – Coefficient of intra-individual and inter-individual biological variation and analytical methods of biochemical parameters

Biochemical parameter	CVbi (%)	CVbg (%)	Analytical method (CAL)	Analytical method (KIKKB)
Lactic acid	27.2	16.7	Lactate oxidation converted to pyruvate	Lactate oxidation converted to pyruvate
Uric acid	8.32 ^e	23.6 ^e	Uricase	Uricase
ALT	19.4	41.6	L-alanine and lactate dehydrogenase	UV with pyridoxal-5'-phosphate
Albumin	3.2	4.75	Bromocresol red	Bromocresol green
Amylase	8.7	28.3	kinetic colorimetric	Ethylidene-p-nitrophenyl-maltoheptaoside G7-pNP
AST	12.3	23.1	L-aspartate and malate dehydrogenase	UV with pyridoxal-5'-phosphate
DB	20.9 ^e	31.1 ^e	Endpoint colorimetric by Doumas	Vanadium oxidation
TB	20.9 ^e	26.6 ^e	Endpoint colorimetric by Doumas	Vanadium oxidation
Ca	1.81 ^e	2.73 ^e	Ortho-cresolphthalein complexone	Ortho-cresolphthalein complexone
Total CPK	22.8	40.0	Coupled enzymatic ^e	N-acetylcysteine activation
Cholesterol	5.18 ^e	17.4 ^e	Endpoint colorimetric	Cholesterol oxidase, esterase, and peroxidase
Creatinine	4.7 ^e	19.0 ^e	Modified Jaffé's kinetic reaction	Kinetic rate of alkaline picrate ^e -IDMS
Fe	26.5	23.2	Iron direct assay by Smith's adaptation	Ferrozine without deproteinization
ALP	6.45	26.1	Kinetic colorimetric ^e	p-nitrophenyl phosphate ^e
Phosphorus	7.67 ^e	10.5 ^e	Phosphomolybdate	Phosphomolybdate method
HDL	5.67 ^e	25.1 ^e	Endpoint colorimetric	Polyanion polymer, direct method

CV: coefficient of variation; CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DB: direct bilirubin; TB: total bilirubin; Ca: calcium; total CPK: total creatine phosphokinase; Fe: iron; ALP: alkaline phosphatase; HDL: high-density lipoprotein.

To calculate the average bias the reports of the EQC were analyzed, provided monthly by the National Quality Control Program (PNCQ), from January to May 2019. It was considered a bias the percentage difference between the result of the control sample analyzed by the laboratory of the university hospital and the mean result of the paired group, represented in the report by the deviation from mean (DFM) (%).

The TE was calculated from the CV and the bias⁽¹⁷⁾, and expressed as a percentage (%). The analytical performance of these parameters was classified as optimal, desirable, and minimal, according to quality specifications based on biological variation.

Taking into account the CV and bias, the sigma (σ) analytical performance of each biochemical analyte was also determined, using the Westgard online calculator⁽¹⁸⁾ for comparison with other studies that use this metric. The sigma was calculated in two ways: using the desirable TE, according to quality specifications based on biological variation, and the allowable TE under the Clinical Laboratory Improvement Amendments (CLIA) criteria, updated in 2019⁽¹⁹⁾. It was considered $\sigma < 3$ as unacceptable analytical performance.

RESULTS

The IQC and EQC data were classified according to analytical performance based on intra-individual and inter-individual

biological variation. The analytical performance analyzed by the CV of each biochemical parameter is shown in **Table 2** and **Figure 1**.

The analytical performance analyzed by the bias of each biochemical parameter is shown in **Table 3** and **Figure 2**.

The analytical performance analyzed by the total error of each biochemical parameter is shown in **Table 4** and **Figure 3**.

The biochemical analytes were also classified according to their sigma level of performance, as shown in **Table 5**.

TABLE 2 – Classification of analytical performance according to the CV of each analyte

Performance	Analytes in the CAL (no.)	KIKKB Institute Analytes (no.)
Great	ALT, TB, Total CPK, Fe, lipase, GGT, TGL (7)	Lactic acid, uric acid, ALT, amylase, TB, total CPK, Fe, LDH, lipase, GGT, TGL, urea (12)
Desirable	Lactic acid, uric acid, amylase, AST, DB, P, K, urea (8)	Albumin, AST, DB, creatinine, P, glucose, K (7)
Minimum	Cholesterol, creatinine, ALP, LDH, glucose (5)	Cholesterol, ALP, HDL (3)
Out of QS	Albumin, Ca, HDL, Mg, TP, Na (6)	Ca, Mg, TP, Na (4)

CV: coefficient of variation; CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications; ALT: alanine aminotransferase; TB: total bilirubin; Total CPK: total creatine phosphokinase; Fe: iron; GGT: gamma-glutamyltransferase; TGL: triglycerides; LDH: lactate dehydrogenase; DB: direct bilirubin; P: phosphorus; K: potassium; AST: aspartate aminotransferase; ALP: alkaline phosphatase; HDL: high-density lipoprotein; Ca: calcium; Mg: magnesium; TP: total proteins; Na: sodium.

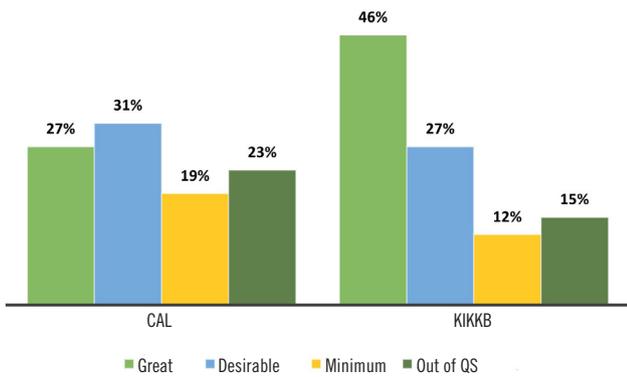


FIGURE 1 – Percentage distribution of analytes according to the analytical performance of the coefficient of variation in the CAL and KIKKB laboratory

CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications.

TABLE 3 – Classification of analytical performance according to the bias of each analyte

Performance	Analytes in the CAL (no.)	KIKKB Institute Analytes (no.)
Great	Amylase, DB, TB, Total CPK, Fe, HDL, LDH, GGT, TGL, urea (10)	Lactic acid, uric acid, ALT, amylase, AST, DB, TB, Fe, ALP, P, LDH, lipase, GGT, TGL, urea (15)
Desirable	Uric acid, ALT, AST, cholesterol, P, lipase (6)	Total CPK, cholesterol, creatinine, HDL, Mg, TP (6)
Minimum	Lactic acid (1)	Glucose (1)
Out of QS	Albumin, Ca, creatinine, ALP, Mg, glucose, K, TP, Na (9)	Albumin, Ca, K, Na (4)

CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications; DB: direct bilirubin; TB: total bilirubin; Total CPK: total creatine phosphokinase; Fe: iron; HDL: high-density lipoprotein.; LDH: lactate dehydrogenase; GGT: gamma-glutamyltransferase; TGL: triglycerides; P: phosphorus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Mg: magnesium; TP: total proteins; Ca: calcium; ALP: alkaline phosphatase; K: potassium; Na: sodium.

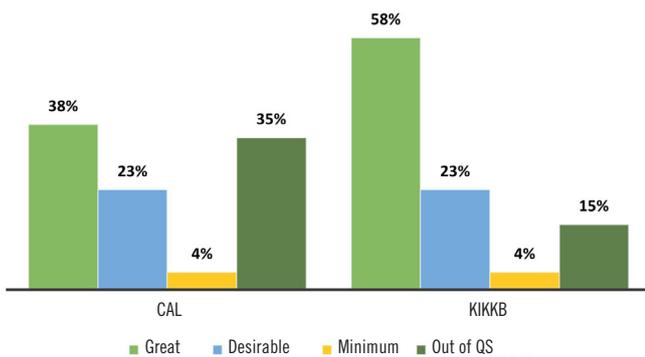


FIGURE 2 – Percentage distribution of analytes according to the analytical performance of bias in the CAL and KIKKB

CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications.

TABLE 4 – Classification of analytical performance according to the TE of each analyte

Performance	Analytes in the CAL (no.)	KIKKB Institute Analytes (no.)
Great	DB, TB, total CPK, Fe, GGT, TGL (6)	Lactic acid, uric acid, ALT, amylase, AST, DB, TB, total CPK, Fe, LDH, lipase, GGT, TGL, urea (14)
Desirable	Lactic acid, uric acid, ALT, HDL, LDH, lipase, amylase, AST, P, urea (10)	Cholesterol, creatinine, ALP, P, HDL (5)
Minimum	Cholesterol, creatinine, glucose (3)	Albumin, Mg, glucose, K, TP (5)
Out of QS	Albumin, Ca, ALP, Mg, K, TP, Na (7)	Ca, Na (2)

TE: total error; CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications; DB: direct bilirubin; TB: total bilirubin; total CPK: total creatine phosphokinase; Fe: iron; GGT: gamma-glutamyltransferase; TGL: triglycerides; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; HDL: high-density lipoprotein; P: phosphorus; ALP: alkaline phosphatase; Mg: magnesium; K: potassium; TP: total proteins; Ca: calcium; Na: sodium.

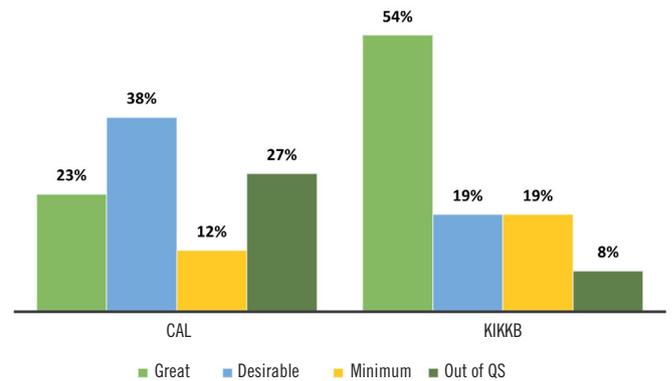


FIGURE 3 – Percentage distribution of analytes according to TE analytical performance in the CAL and KIKKB

CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications; TE: total error.

DISCUSSION

Knowing the causes of errors can help considerably in recognizing errors and speeding up resolution while they are still occurring. When errors are identified by the IQC, the patient results should be delayed and re-analyzed after corrective action⁽²⁰⁾. Likewise, the bias observed in the EQC should be used to assess the possible impact on the outcome of previously released results.

The CV or random error, expressed as a percentage, represents the SD of the mean and is related to the inaccuracy of the method. When we perform repeated measurements of a stable material under identical conditions (the same operator using the same types of equipment and reagents), we get a series of different results that

TABLE 5 – Analytical performance according to the sigma metric of each analyte using the allowable TE under CLIA criteria and the desirable TE according to biological variation

Performance	Analytes in the CAL (No.; %)		KIKKB Institute Analytes (No.; %)	
	With desirable TE based on biological variation	With TE of the CLIA criteria	With desirable TE based on biological variation	With TE of the CLIA criteria
Great > 6	Total CPK, Fe, GGT, TGL (4; 15)	(0; 0)	Lactic acid, uric acid, ALT, amylase, TB, total CPK, Fe, LDH, lipase, GGT, TGL, urea (12; 46)	Uric acid, amylase, TB, Ca, Fe, LDH, Mg, GGT, TGL (9; 39)
Desirable 4-5.9	Uric acid, ALT, amylase, DB, TB, lipase, urea (7; 27)	TB, total CPK, P, Na (4; 17)	AST, DB (2; 8)	Albumin, AST, Total CPK, ALP, HDL, glucose, K, TP (8; 35)
Minimum 3-3.9	P (1; 4)	Uric acid, amylase, Ca, Fe, HDL, LDH, GGT, TGL (8; 35)	P, glucose (2; 8)	ALT, creatinine, P, urea (4; 17)
Out of QS < 3	Lactic acid, albumin, AST, Ca, cholesterol, creatinine, ALP, HDL, LDH, Mg, glucose, K, TP, Na (14; 54)	ALT, albumin, AST, cholesterol, creatinine, ALP, Mg, glucose, K, TP, urea (11; 48%)	Albumin, Ca, cholesterol, creatinine, ALP, HDL, Mg, K, TP, Na (10; 38)	Cholesterol, Na (2; 9)

TE: total error; CLIA: Clinical Laboratory Improvement Amendments; CAL: Clinical Analysis Laboratory; KIKKB: Slovenian Institute of Clinical Chemistry and Biochemistry; QS: quality specifications; total CPK: total creatine phosphokinase; Fe: iron; GGT: gamma-glutamyltransferase; TGL: triglycerides; ALT: alanine aminotransferase; DB: direct bilirubin; TB: total bilirubin; P: phosphorus; AST: aspartate aminotransferase; Ca: calcium; ALP: alkaline phosphatase; HDL: high-density lipoprotein; LDH: lactate dehydrogenase; Mg: magnesium; K: potassium; TP: total proteins; Na: sodium.

are due to the random variation that occurs in every measurement process. This random error can be reduced by optimizing processes, but never eliminated⁽²⁰⁾. Regarding the CV, the KIKKB Institute presented a greater number of analytes with optimal performance, reaching 46% of the tests, while only 27% presented this performance in the CAL. The analytes that diverged in this criterion were amylase, lactic acid, uric acid, and urea, with desirable performance; and LDH, with minimal performance in the CAL. However, for the most part, they differed on only one level of performance.

Of all 26 analytes, 17 had the same analytical performance for CV in both laboratories. The CAL presented six analytes outside the QS for CV, being four out of the specifications in the KIKKB Institute: Ca, Mg, TP and Na. The inaccuracy, characterized by random variation in results, should be related to: different reagents batches used over the period evaluated, different calibrations of the analytical system, deterioration of the reagent water quality, timeout of filter replacement, noise electronics, unstable reading due to electrical problems, carry-over effect, needle contamination, system air bubbles, etc.⁽²⁰⁾.

As observed for CV, the bias also had more analytes outside the QS in the CAL (35%) than in KIKKB Institute (15%). Again, all KIKKB analytes outside the QS were also outside the QS in the CAL: albumin, Ca, Na and K.

Although the proficiency testings are different, the observed bias was similar between laboratories. The reasons of bias are usually related to improper sample transportation or storage, uncalibrated equipment, and deterioration of reagents and equipment⁽¹⁹⁾. In addition, the use of proficiency testing data may

overestimate the bias, which is most likely due to the random errors that also occur in these results and the rarity of the execution of the tests⁽²¹⁾. Some authors argue that the standardization of analytical methods could reduce the bias because comparing different methods becomes complicated^(22, 23). Another limitation of assessing bias through proficiency testing results is that they are not metrologically traceable like reference materials⁽²¹⁾.

In 2016, a study by Weykamp *et al.* (2017)⁽¹³⁾ in Italy, Netherlands, Portugal, Spain, and the United Kingdom compared the TE between different equipment for the main biochemical analytes and classified them according to the QS based on the intra-individual and inter-individual biological variation of the Westgard database. The minimum quality specifications for TE were not met for Na, Chlorine (Cl), and Ca in any country and in any equipment, including Dimension[®] and Advia[®] devices⁽¹³⁾, used respectively in the CAL and KIKKB Institute. These results confirm the unmet QS for the Na and Ca analytes in both laboratories evaluated in this study, even when the intra-individual and inter-individual biological variation of the EuBIVAS study is employed. The TP and ALP tests, on the other hand, which were outside the QS in relation to TE in the CAL, showed minimum performance in Dimension[®] device, according to Weykamp *et al.* (2017)⁽¹³⁾.

The CAL analytes that were out of the QS for CV, bias, and TE were: albumin, Mg, TP, Na, and Ca, representing 19% of the analytes. Therefore, these would be the analytes with the topmost significance for assaying the process and taking corrective conduct. Among these parameters, the Na test was one of the most requested in the laboratory in 2017.

The Ca and Na results demonstrated CV, bias, and TE outside the QS in both laboratories. The electrolyte analysis is quite sensitive to contaminants in the equipment hoses, filter saturation, and, consequently, the quality of the reagent water⁽²⁴⁾. Furthermore, these analytes have a very narrow biological variation, the lowest of all the biochemical analytes evaluated in this study. The intra-individual biological variation is around 0.5% and 2%, and the inter-individual, 1.5% and 3%, for Na and Ca, respectively, according to the EuBIVAS⁽¹¹⁾. According to the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Database, the studies of biological variation of Na and Ca are from the years 1970 to 2018, being the EuBIVAS study the most recent to employ the Advia 2400[®] equipment. Thus, it is necessary to think of more adequate and less strict quality goals for analytes with a small biological variation or more robust methods capable of presenting greater accuracy.

Even using the Westgard biological variation database to calculate the quality goals, the Ca and Na analytes do not meet the specifications of any criteria (CV, bias, or TE) in both laboratories (results not shown). In the Westgard database, CVbi is 2.1% for Ca and 0.6% for Na, while decreasing to 1.81% and 0.53%, respectively, in the EuBIVAS study.

The specification of quality based on biological variation is advantageous because it sets demanding and objective goals that encourage the constant search for improvement. However, the goals may not be possible to achieve with the methods currently used⁽²⁴⁾. Some authors consider the use of biological variation very subjective for analytes with very narrow biological variation, such as Na, Cl, and albumin. According to Ricos *et al.* (2009)⁽²⁵⁾, only 10% of laboratories participating in the EQC by way of SEQC program meet the quality specifications of TE for albumin, 20% for HDL cholesterol, 50% for Na and Cl, and 70% for Ca, when to be calculated by biological variation. The authors suggest that the goals can be achieved by laboratories. However, the data used in that study are from laboratories that participate in the same specific proficiency testing without a specification of which self-analyzer was used.

Analytical performance in relation to TE was more conflicting among laboratories for ALP test, which demonstrated desirable performance in KIKKB Institute and was outside the QS in the CAL. In the study by Weykamp *et al.* (2017)⁽¹³⁾, both Dimension[®] and Advia[®] equipment demonstrated minimum performance in relation to this analyte. Although considered to be Siemens equipment, Dimension[®] and Advia[®] are originally from different companies: Bayer and Dade Behringer, respectively. Thus, identical results were not expected; it is only assumed that they have the same quality standard for maintenance and reagents.

The analysis of external quality control reports from the CAL demonstrates that a failure in a cycle influences overall analytical performance regardless of the size of the bias. For example, the analytes ALT, glucose, TP, Fe, K, and Na were considered unacceptable in one cycle, while ALP and lipase were considered unacceptable in two (results not shown). Of these analytes, those that were out of the QS based on biological variation were: ALP, glucose, PT, K, and Na. Lipase and ALT which achieved the desirable targets, and Fe showed excellent analytical performance.

Regarding TE for the CAL, 77% of the analytes were in the QS when based on Westgard databank, and 73% when based on EuBIVAS data. As for KIKKB Institute, there was no difference in the number of analytes in the QS when compared to the use of Westgard and EuBIVAS databases. The assessment of analytical performance, comparing the calculated QS with the biological variation of the Westgard and EuBIVAS database, showed no significant difference in the performance classification of the analytes, only one level change occurs in one of the criteria (CV, bias, or TE) for some analytes (results not shown). Overall, the EuBIVAS presents lower intra-individual and higher inter-individual biological variation data. Of the 15 analytes that have biological variation in both databases, 93% have lower CVbi in the EuBIVAS compared to the Westgard database, and 60% have higher CVbg in the EuBIVAS compared to the Westgard database. Thus, most analytes rose one performance level relative to CV when they were ranked using the Westgard database (results not shown).

Another way to assess quality is to employ the sigma metric since it allows the comparison of quality among different processes⁽²⁶⁾. Using this criterion, the analytical performance of the CAL was inferior to KIKKB Institute, and the percentage of analytes with sigma > 3 in KIKKB was 61.5%, while in the CAL had less than half (46.2%) of the analytes with this performance, considering the desirable TE for the calculation of the metric. In both laboratories, the number of parameters outside the QS, that is, with unacceptable sigma (< 3), increased in relation to quality goals based on biological variation. When using the allowable TE under CLIA criteria to calculate sigma, the number of analytes outside the QS decreased for the CAL and KIKKB: from 54% to 48% and from 38% to 9%, respectively.

Berlitz and Haussen (2005)⁽²⁶⁾ found in the Advia 1650[®] equipment the lowest indexes of the sigma metric (< 2) for the transaminase enzymes (AST and ALT) and the highest for TGL, uric acid, Na, and K (> 6), using the allowable TE based on the CLIA criteria. In our study, when the desirable TE was used, both CAL and KIKKB Institute presented the Na and K analytes with unacceptable sigma values. In KIKKB Institute, TGL and uric acid also had sigma > 6, and in the CAL only TGL. Regarding

transaminases, ALT performed > 4 in the CAL and KIKKB. On the other hand, AST got sigma 5 in KIKKB and unacceptable in the CAL. An improvement in the performance of the parameters in the Advia[®] equipment is observed when comparing the 1650 and 1800 models, since, when using the TE based on the CLIA criteria, only two analytes present unacceptable performance in the KIKKB (Advia[®] 1800).

When a method gets sigma > 6 , strict IQC rules are not required⁽²⁷⁾. This is the case for the analytes creatinine, TGL, total CPK, amylase, and AST in the study by Singh *et al.* (2011)⁽²⁷⁾, who obtained sigma > 6 using the allowable TE based on the CLIA criteria; the urea test had low performance (sigma < 3). Compared to our data based on the desirable TE, only TGL, amylase, and total CPK achieved similar performance in the CAL and KIKKB. The creatinine test had unacceptable performance both in KIKKB and in CAL, which also had AST with sigma performance level < 3 . The urea test had sigma 4 in the CAL and sigma > 6 in the KIKKB. Considering the results based on TE of the CLIA criteria, the urea test also has a sigma < 3 in the CAL and a minimum performance in the KIKKB Institute.

Analytes with sigma performance level < 3 deserve special attention and must be analyzed by three levels of control in the IQC twice a day, applying the Westgard rules $1_{3s}/2_{2s}/R_{4s}/4_{1s}$ ^(4,15). In order to improve the sigma of these parameters, better methodologies and equipment can be implemented. In the laboratory where the study by Mao *et al.* (2018)⁽¹⁵⁾ was analyzed, the analytes Na, chloride, K, and urea had unacceptable sigma level (< 3). Both in CAL and KIKKB, Na and K had sigma < 3 based on the desirable TE, but they differ when compared to the TE based on the CLIA criteria, also used in the study by Mao *et al.* (2018)⁽¹⁵⁾.

On the other hand, analytes with sigma performance between 3 and 4 must be evaluated by two levels of control in IQC, also twice a day, with the same Westgard rules applied when sigma is < 3 ^(4, 15). This would be applied to P, which had sigma between 3 and 4 in the CAL and KIKKB, and glucose, which had sigma between 3 and 4 in KIKKB when calculated from the desirable TE. The albumin test, which demonstrated this performance in the study by Mao *et al.* (2018)⁽¹⁵⁾ carried out in the Beckman Coulter[®] AU5800 equipment, presented unacceptable sigma in the CAL considering the desirable TE and the CLIA criteria.

For analytes with sigma performance level between 4 and 6 and > 6 , is recommended the evaluation by two levels of control in IQC, once a day, using the rule $1_{2.5s}$ and 1_{3s} , respectively^(4,15).

Another piece of equipment from Siemens, the Atellica[®] CH 930, performed better than Dimension[®] and Advia[®], also evaluated in the study by Fasano *et al.* (2019)⁽²⁸⁾, with all 13 biochemical

parameters analyzed with sigma > 3 . However, to calculate the sigma metric, the study used the allowable TE according to CLIA criteria, which was similar or less rigorous than the allowable TE based on biological variation in most analytes. The authors themselves consider further constant updating of biological variation data and the recent procedures for standardizing these data for use in the calculation of allowable TE⁽²⁸⁾.

Evaluating the analytical performance by the sigma metric of eight enzymes (ALT, AST, GGT, amylase, lipase, ALP, CPK, and LDH) in the Cobas[®] 8000 c702 equipment (Roche, Germany), none of them presented unacceptable sigma⁽²⁹⁾. Of these enzymes, LDH, ALP, and AST had sigma < 3 in the CAL when considering the desirable TE, and ALT, AST, and ALP when considering the TE of the CLIA criteria. In KIKKB, only ALP presented sigma level < 3 when considering the desirable specifications for TE and no enzyme when considering the TE of the CLIA criteria. However, the sigma metric used in the study by Zeng *et al.* (2019)⁽²⁹⁾ was calculated based on the QS of the EFLM and not on the biological variation nor on the CLIA criteria as it happened in our study.

The allowable TE according to the CLIA criteria was more rigorous for analytes with greater biological variation compared to the desirable specifications for TE based on biological variation and less strict for analytes with a very narrow biological variation range, as is the case for Na and Ca.

According to Mao *et al.* (2018)⁽¹⁵⁾, the sigma metric serves as a self-assessment method, without the need to compare the performance with other laboratories, to plan the number and frequency of IQC inspections, and to produce accurate results. Still, the sigma metric proved to be more rigorous in evaluating the analytical performance of biochemical parameters when compared to the QS based on biological variation.

In order to ensure the quality of laboratory results, regardless of the method to be used, performance evaluation should be carried out whenever there is any change in the analytical process, preferably once a year.

CONCLUSION

In general, the analytical performance of the CAL was considered acceptable, as it presented results similar to those of the KIKKB Institute, which is 10 times larger and performs five more times IQC. In addition, only five (19%) of the analyzed analytes performed outside the QS on all criteria (CV, bias, and TE). The tests that should be targeted for improvement measures are: albumin, Ca, Mg, TP and Na. The sigma metric proved to

be more rigorous in assessing the analytical performance of biochemical parameters compared to the quality specifications based on biological variation, as it increased to 11, the analytes (48%) outside the QS.

The non-achievement of the quality goals was not laboratory-specific, as the performance of different parameters between the two laboratories and the comparative study is similar.

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