RESEARCH ARTICLE

Quality specifications of routine clinical chemistry methods based on sigma metrics in performance evaluation

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Introduction: Sigma metrics were applied to evaluate the performance of 20 routine chemistry assays, and individual quality control criteria were established based on the sigma values of different assays.

Methods: Precisions were expressed as the average coefficient variations (CVs) of long-term two-level chemistry controls. The biases of the 20 assays were obtained from the results of trueness programs organized by National Center for Clinical Laboratories (NCCL, China) in 2016. Four different allowable total error (TEa) targets were chosen from biological variation (minimum, desirable, optimal), Clinical Laboratory Improvements Amendments (CLIA, US), Analytical Quality Specification for Routine Analytes in Clinical Chemistry (WS/T 403-2012, China) and the National Cholesterol Education Program (NECP).

Results: The sigma values from different TEa targets varied. The TEa targets for ALT, AMY, Ca, CHOL, CK, Crea, GGT, K, LDH, Mg, Na, TG, TP, UA and Urea were chosen from WS/T 403-2012; the targets for ALP, AST and GLU were chosen from CLIA; the target for K was chosen from desirable biological variation; and the targets for HDL and LDL were chosen from the NECP. Individual quality criteria were established based on different sigma values.

Conclusions: Sigma metrics are an optimal tool to evaluate the performance of different assays. An assay with a high value could use a simple internal quality control rule, while an assay with a low value should be monitored strictly.

KEYWORDS

allowable total error, quality control, sigma metrics

1 | INTRODUCTION

Sigma metrics methodology is a process-based quality management and improvement program developed by Motorola in the early 1980s, which soon became famous and was used by other manufacturers and continues to be used today to drive quality improvements and standards. In the 21st century, sigma metrics were introduced into clinical laboratories by David Nevalainen and Lucia Berta.¹ Quality performance can be assessed by sigma value, where 3 sigma is the minimum performance, and 6 sigma is the world-class quality, such that 99.99966% of results are error free, corresponding to 3.4 defects per million opportunities.²

In many Chinese clinical laboratories, the Westgard multiplication rules (1_{3s} , 2_{2s} , R_{4s} , 4_{1s} , 10_x) have been widely used in clinical chemistry. In this method, there may be more false rejection, waste of quality control materials and prolonged test awaiting time (TAT), whereas the sigma metrics approach allows a laboratory to take a broader view of the data, joining estimates of bias and imprecision into a practical judgment on the clinical usefulness of the method's quality control criteria.^{3,4} Thus, it is useful and simple to access and choose optimal internal quality control criteria based on sigma metrics.

The objective of this study is to evaluate the performances of 20 routine chemistry assays on a Beckman Coulter AU5800 instrument in

our laboratories and try to establish individual internal quality control criteria based on sigma metrics values.

2 | MATERIALS AND METHODS

2.1 | Analyzer and assays

An AU5800 (Beckman Coulter, Brea, CA, USA) analysis system was used to detect 20 routine chemistry assays: alkaline phosphatase (ALP, International Federation Of Clinical Chemistry [IFCC] reference method), alanine aminotransferase (ALT, IFCC reference method modified without pyridoxal phosphate), amylase (AMY, IFCC reference method), aspartate aminotransferase (AST, IFCC reference method modified without pyridoxal phosphate), calcium (Ca, Arsenazo III method), cholesterol (CHOL, cholesterol oxidase and peroxidase method), creatine kinase (CK, IFCC reference method), creatinine (Crea, uncompensated Jaffe reaction method), gamma-glutamyltransferase (GGT, IFCC reference method), glucose (Glu, hexokinase method), high-density lipoprotein cholesterol (HDL, accelerator selective detergent method), potassium (K, ion selective electrodes method), lactate dehydrogenase (LDH, IFCC reference method), low-density lipoprotein cholesterol (LDL, liquid selective detergent method), magnesium (Mg, Xylidyl blue method), sodium (Na, ion-selective electrodes method), triglyceride (TG, glycerol phosphate oxidase and peroxidase method), total protein (TP, Biuret method), uric acid (UA, uricase and peroxidase method) and urease (Urea, kinetic assay method). All the reagents are Beckman Coulter original except HDL and LDL from Sekisui (Osaka, Japan) and AMY from Leadman (Beijing, China).

2.2 | Controls

Two-level internal quality controls (SYNCHRON Control, Beckman Coulter, US) were analyzed, and it was determined whether the results were within acceptable limits and could be reported. The target values in high level (Lot. M407083) were ALP 290 U/L, ALT 348 U/L, AMY 958 U/L, AST 372 U/L, Ca 3.26 mmol/L, CHOL 5.10 mmol/L, CK 678 U/L, Crea 621.5 μ mol/L, GGT 433 U/L, Glu 23.26 mmol/L, HDL 1.63 mmol/L, K 6.95 mmol/L, LDH 407 U/L, LDL 2.67 mmol/L, Mg 1.45 mmol/L, Na 149.20 mmol/L, TG 1.54 mmol/L, UA 708 μ mol/L, TP 81.50 g/L, Urea 23.22 mmol/L; the target values in low level (Lot. M407082) were ALP 166 U/L, ALT 186 U/L, AMY 520 U/L, AST 205 U/L, Ca 2.54 mmol/L, CHOL 3.75 mmol/L, CK 371 U/L, Crea 361.50 μ mol/L, GGT 237 U/L, Glu 12.97 mmol/L, HDL 1.15 mmol/L, K 4.69 mmol/L, LDH 236 U/L, LDL 1.88 mmol/L, Mg 0.98 mmol/L, Na 135.50 mmol/L, TG 1.13 mmol/L, UA 445 μ mol/L, TP 59.80 g/L, Urea 13.05 mmol/L. Prior to analysis, the control samples were allowed to come to room temperature and air bubbles were removed. Acceptance was determined by the use of the Westgard rules (1_{3s} , 2_{2s} , R_{4s} , 4_{1s} , 10_x).⁵

2.3 | Precision and bias

Precision, expressed as the coefficient of variation (CV), was calculated by 9 months' CV results of two-level quality controls. The

arithmetic average of the CVs was used. The 20 chemistry assays were part of the trueness programs organized by the National Center for Clinical Laboratories (NCCL) in 2016 in China as it was showed in Supplemental Material (Table S1). This program includes NCCL-C-C15: metabolite/total protein trueness verification (Crea, Glu, TP, UA and Urea), NCCL-C-C16: lipid trueness verification (CHOL, HDL, LDL and TG), NCCL-C-C17: enzyme trueness verification (ALP, ALT, AMY, AST, CK, GGT and LDH), and NCCL-C-C19: electrolyte trueness verification (Ca, K, Mg and Na). Reference samples were transported to laboratories on dry ice and were checked and stored according to the instructions. Individual assays' biases were calculated and reported by NCCL, and the arithmetic average of these biases was used in the calculation of sigma metrics.

2.4 | Allowable total error (TEa)

Definition of the TEa requirements for the different clinical chemistry assays should be the starting point for the managing quality. Four different TEa targets were used in this study: TEa based on biological variation (minimum, desirable, optimal, which can be searched in <http://www.westguard.com>), TEa selected from the Clinical Laboratory Improvements Amendments (CLIA) requirements, from the Analytical Quality Specification for Routine Analytes in Clinical Chemistry (WS/T 403-2012, China) requirements and from the National Cholesterol Education Program (NECP), which had specifications for HDL and LDL.^{6,7}

Using biological variation data, TEa specifications can be derived from intra- and inter-individual biological variation as the equations below (CV_I =within-subject biological variation, CV_G =between-subject biological variation)⁸:

Minimum: $CV < 0.75 CV_I$, Bias $< 0.375 (CV_I^2 + CV_G^2)^{0.5}$, TEa $< 1.65 * 0.75 CV_I + 0.375 (CV_I^2 + CV_G^2)^{0.5}$

Desirable: $CV < 0.5 CV_I$, Bias $< 0.25 (CV_I^2 + CV_G^2)^{0.5}$, TEa $< 1.65 * 0.5 CV_I + 0.25 (CV_I^2 + CV_G^2)^{0.5}$

Optimal: $CV < 0.25 CV_I$, Bias $< 0.125 (CV_I^2 + CV_G^2)^{0.5}$, TEa $< 1.65 * 0.25 CV_I + 0.125 (CV_I^2 + CV_G^2)^{0.5}$

2.5 | Sigma calculation

Sigma metrics were calculated using the standard equation below:

$$\text{Sigma metric} = (\text{TEa} - |\text{Bias}|) / \text{CV}^9$$

The performances of 20 assays were evaluated, and the optimal TEa targets that reflected the real performances of these assays were ultimately chosen.

3 | RESULTS

The performances of 20 assays in the AU5800 analysis system and the different sigma metric values were calculated based on different TEa targets, and the results are shown in Table 1.

Using biological variation minimum targets, ten assays were higher than 6 sigma, four assays were between 4 and 6 sigma, six assays

TABLE 1 Performances of the 20 assays in the AU5800 analysis system and the sigma metric values calculated based on different TEAs

			Biological variation						CLIA		WS/T 403-2012		NCEP	
			Minimum		Desirable		Optimal							
Assay	CV%	Bias%	TEa%	Sigma	TEa%	Sigma	TEa%	Sigma	TEa%	Sigma	TEa%	Sigma	TEa%	Sigma
ALP	1.76	19.63	18.06	−0.89	12.04	−4.32	6.02	−7.74	30.00	5.90	18.00	−0.93		
ALT	1.34	5.56	41.22	26.54	27.48	16.32	13.74	6.09	20.00	10.75	16.00	7.77		
AMY	1.33	4.60	21.87	13.01	14.58	7.52	7.29	2.03	30.00	19.14	15.00	7.84		
AST	1.58	11.17	25.04	8.78	16.69	3.50	8.35	−1.79	20.00	5.59	15.00	2.42		
Ca	1.39	0.93	3.82	2.08	2.55	1.16	1.27	0.25			5.00	2.92		
CHOL	1.55	4.97	13.52	5.50	9.01	2.60	4.51	−0.30	10.00	3.24	9.00	2.60		
CK	1.52	3.45	45.48	27.62	30.32	17.66	15.16	7.70	30.00	17.45	15.00	7.59		
Crea	1.57	15.66	13.31	−1.49	8.87	−4.31	4.44	−7.13	15.00	−0.42	12.00	−2.33		
GGT	1.86	1.40	33.17	17.12	22.11	11.16	11.06	5.20	20.00	10.02	11.00	5.17		
Glu	1.61	2.43	10.44	4.97	6.96	2.81	3.48	0.65	10.00	4.69	7.00	2.83		
HDL	2.65	2.60	17.44	5.60	11.63	3.41	5.81	1.21					13.00	3.92
K	0.71	1.61	8.41	9.52	5.61	5.60	2.80	1.68			4.00	3.35		
LDH	2.20	2.60	17.03	6.56	11.35	3.98	5.68	1.40	20.00	7.91	11.00	3.82		
LDL	2.98	4.83	17.84	4.37	11.90	2.37	5.85	0.38					12.00	2.41
Mg	1.61	1.72	7.21	3.41	4.81	1.92	2.40	0.43	25.00	14.47	15.00	8.25		
Na	0.54	1.40	1.09	−0.57	0.73	−1.24	0.36	−1.92			4.00	4.84		
TG	1.74	1.72	38.98	21.46	25.99	13.98	12.99	6.50	25.00	13.41	14.00	7.07		
UA	1.44	1.46	17.95	11.45	11.97	7.30	5.98	3.14	17.00	10.79	12.00	7.32		
TP	1.21	1.22	5.45	3.48	3.63	1.99	1.82	0.49	10.00	7.24	5.00	3.11		
Urea	1.50	0.30	23.33	15.38	15.55	10.19	7.78	5.00	9.00	5.81	8.00	5.15		

CLIA, Clinical Laboratory Improvements Amendments; NECP, National Cholesterol Education Program; CV, coefficient of variation; TEa, Allowable Total Error.

were between 3 and 4 sigma, and four assays (ALP, Ca, Crea and Na) were lower than 3 sigma. Using desirable targets, seven assays were higher than 6 sigma, one assay was between 4 and 6 sigma, three assays were between 3 and 4 sigma, and nine assays were lower than 3 sigma. Using optimal targets, three assays (ALT, CK and TG) were higher than 6 sigma, two assays were between 4 and 6 sigma, two assays were between 3 and 4 sigma, and fourteen assays were lower than 3 sigma.

Sixteen assays were evaluated using CLIA requirements. Ten assays were higher than 6 sigma, four assays were between 4 and 6 sigma, one assay was between 3 and 4 sigma, and only Crea was lower than 3 sigma. Eighteen assays were evaluated using the WS/T 403-2012 targets. Six assays were higher than 6 sigma, three assays were between 4 and 6 sigma, three assays were between 3 and 4 sigma, and six assays were lower than 3 sigma. There are general recommendations for the performance of HDL and LDL from NCEP. When using these TEa targets, HDL was higher than 3 sigma, while LDL was lower than 3 sigma.

The optimal TEa targets of the 20 assays should be chosen based on their performances in the laboratory, and the internal quality control criteria were established based on specific sigma metric values (Table 2). Assay performance chart for sigma metrics¹⁰ was used to

evaluate the performances of these assays (Figure 1), which could be drawn online (<http://www.clinet.com.cn/sigmapv/>). The assay performances were divided into world class (≥ 6 sigma), excellent (≥ 5 sigma and < 6 sigma), good (≥ 4 sigma and < 5 sigma), marginal (≥ 3 sigma and < 4 sigma), poor (≥ 2 sigma and < 3 sigma) and unacceptable (< 2 sigma).

4 | DISCUSSION

Sigma metrics can be used in many ways in the clinical laboratory, such as verifying and monitoring the performances of new assays, establishing individual quality criteria and quality improvement programs.¹¹⁻¹³ In this study, we used sigma metrics to evaluate the quality performances of 20 routine chemistry assays tested using the AU5800 instrument in our laboratory. Four different TEa targets were used to help calculate the sigma values.

In the statements of the Stockholm conference in 1999,^{14,15} data based on components of biological variation are level 2a of the hierarchy for calculating TEa targets; in the Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine, biological variation are model 2 for setting performance specifications.¹⁶ It was divided into three

TABLE 2 Optimal TEa targets of the 20 assays and internal quality control (IQC) criteria used based on sigma values

Assays	Optimal TEa targets %	Sigma value	IQC criteria (N=2 rules)
ALP	30.00 (CLIA'88)	5.90	1 _{3S}
ALT	16.00 (WS/T 403-2012)	7.77	1 _{3S}
AMY	15.00 (WS/T 403-2012)	7.84	1 _{3S}
AST	20.00 (CLIA'88)	5.59	1 _{3S}
Ca	5.00 (WS/T 403-2012)	2.92	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
CHOL	9.00 (WS/T 403-2012)	2.60	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
CK	15.00 (WS/T 403-2012)	7.59	1 _{3S}
Crea	12.00 (WS/T 403-2012)	-2.33	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
GGT	11.00 (WS/T 403-2012)	5.17	1 _{3S} /2 _{2S}
Glu	10.00 (CLIA'88)	4.69	1 _{3S} /2 _{2S} /R _{4S} /4 _{1S}
HDL	13.00 (NECP)	3.92	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
K	5.61 (biological variation, desirable)	5.60	1 _{3S}
LDH	11.00 (WS/T 403-2012)	3.82	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
LDL	12.00 (NECP)	2.41	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
Mg	15.00 (WS/T 403-2012)	8.25	1 _{3S}
Na	4.00 (WS/T 403-2012)	4.84	1 _{3S} /2 _{2S} /R _{4S} /4 _{1S}
TG	14.00 (WS/T 403-2012)	7.07	1 _{3S}
TP	5.00 (WS/T 403-2012)	3.11	1 _{3S} , 2 _{2S} , R _{4S} , 4 _{1S} , 10 _x
UA	12.00 (WS/T 403-2012)	7.32	1 _{3S}
Urea	8.00 (WS/T 403-2012)	5.15	1 _{3S} /2 _{2S}

classes: minimum, desirable and optimal. Based on the sigma values, we found that the optimal TEa targets are so tight that 70% (14/20) of assays were below 3 sigma, and only three assays (ALT, CK and TG) reached the world-class level. It seems that this criterion is unsuitable for use in routine chemistry. The desirable TEa may be better, and the minimum TEa provides the highest sigma metrics, such that only four assays (ALP, Ca, Crea and NA) were below the minimum performance request.

Sixteen assays' TEa targets were from CLIA. Crea is the only assay below 3 sigma. The bias of Crea is 15.66%, which exceeds the NCCL standard. The reason for this poor performance is that the Crea

FIGURE 1 Assay performance chart for Sigma metrics. Sigma metrics calculated using optimal TEa targets from biological variance, CLIA, WS/T 403-2012 and NECP. Dots represent different assays

method used in our laboratory is the uncompensated Jaffe reaction, which cannot be traced to the reference method. Thus, the result suggests that a more traceable method should be used.¹⁷ WS/T 403-2012 is the standard requirements for the analytical quality specification for routine analytes in clinical chemistry in China.¹⁸ Thus, we also used these TEa targets to calculate the sigma metrics of these assays. There were six assays below 3 sigma (ALP, AST, Ca, CHOL, Crea and GLU). WS/T 403-2012 has tighter GLU and AST TEa, so the sigma is low. The bias of ALP is 19.63%, and the variance may be caused by the value of the Beckman coulter ALP master calibrator. CHOL's bias is 4.97%, slightly higher than the criterion, indicating that steps should be taken to improve the performance of this assay in our laboratory. In 1995, NECP established series criteria for the analysis of Cholesterol, including HDL and LDL. Thus, in this study, we also use NECP's TEa targets to calculate the sigma value. HDL had better results than LDL, which suggested that LDL's performance should be monitored more strictly.

Although there are many TEa targets for routine chemistry, the optimal ones should be established depending on the conditions and requirements of the individual laboratory. The requirements should be neither too low nor too high. In our laboratory, the first choice standard is WS/T 403-2012, which is the standard set of requirements in China. If the standard is too strict, other targets could be used (ALP, AST, Glu from CLIA; K from biological variation desirable; HDL and LDL from NECP).

In this study, we try to establish individual TEa targets for different assays. Because there is no obvious rule to obey, we evaluated four different requirements and chose the final targets based on the performances of these 20 assays in our laboratory. One weakness of this study is in the calculation of the sigma metrics. Precisions were calculated from two-level internal quality controls, and biases were calculated from two or four concentrations of the trueness programs samples. The different concentrations may influence the results of the sigma metrics.

We only evaluated these 20 assays in our laboratory because these 20 assays were part of the trueness programs organized by NCCL, and thus the bias results were closer to the truth. Other assays which took part involved in the external quality assessment program in China may also be evaluated later based on the bias results.

Before using sigma metrics to evaluate the performance of these assays, westgard rules (1_{3s} , 2_{2s} , R_{4s} , 4_{1s} , 10_x) were used for different assays in our laboratory. This method may lead to more false rejection and waste of quality control materials, as well as prolonged test awaiting time (TAT). Therefore, in this study we also established individual internal quality control criteria based on the sigma values. As shown in Figure 1, certain assays with world-class or excellent performances (ALP, ALT, AMY, AST, CK, K, Mg, TG and UA) only used 1_{3s} , while assays with marginal, poor or unacceptable performances were maintained under the strict control rules as before.

Ultimately, sigma metrics methodology is an optimal tool to evaluate the performances of different assays. Assay with high sigma value could use simple internal quality control rules to reduce false rejection, while assays with low values should be monitored strictly. Furthermore, assays with poor performance should be reviewed, and perhaps different methods could be considered to replace them.

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SUPPORTING INFORMATION

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