

Evaluation of Analytical Performance in Clinical Biochemistry Laboratory in India Using Six Sigma Methodology

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ABSTRACT

Introduction: Internal and external quality controls (QCs) used in the laboratory are effective in detecting analytical errors. However, they cannot quantify the number of errors. Six sigma can be used to objectively evaluate the performance of analytical methods. Hence, we have evaluated the analytical performance of 19 parameters using six sigma methodology.

Materials and methods: Quality control data were collected over a period of 6 months—from January to June 2016—and sigma metric was calculated. Parameters showing sigma metrics of ≤ 3 were further analyzed between July and September 2016 by applying the suggested rules from Unity Real Time (URT) software.

Results: Gamma-glutamyl transferase (GGT) Level (L) 2 showed the highest value of sigma (13.22). Total bilirubin was found to have the highest sigma values at both control levels (7.15 and 9.49 at L1 and L2, respectively). Sigma value of ≥ 4 was observed across all control levels for anti-TPO, CK-MB, potassium, PSA, and TSH. L1 of alpha feto protein (AFP) and L2 of Troponin I had sigma value of ≤ 3 . We have obtained sigma value of ≤ 3 for all levels of remaining analytes. Among these, L1 of AFP showed a significant improvement in sigma after the application of suggested rules (2.5 to 9.3).

Conclusion: The sigma value for a test is a good indication of its process capability because it considers both bias and imprecision. Unfortunately, most clinical laboratory tests are below six sigma processes. It is imperative to implement appropriate QC strategies for the judicious use of quality control.

Keywords: Quality control, Root cause analysis, Six sigma, Total error, Westgard rules.

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INTRODUCTION

Quality control (QC) is used in medical laboratory to monitor the performance of analytical process.¹ Quality in the lab can be evaluated by using a combination of internal quality control (IQC) and proficiency testing (PT).

A combination of IQC and PT program is effective in detecting analytical errors. However, the number of errors (or defects) in the laboratory cannot be quantified by reviewing IQC and PT programs. The quantifiable parameters obtained from IQC/PT programs can be used to calculate sigma metrics which would provide a quantifiable measure of errors/defects in the laboratory. The advantage of sigma metric is that in one number, it neatly summarizes a characteristic of multiple key analytical performance characteristics.²

In a collective opinion paper on findings of the 2010 convocation of experts on laboratory quality, Cooper et al. suggested the use of sigma to decide QC frequency. They suggested that the tests should be divided into three groups as follows:²

- $>6\sigma$ (excellent tests)—evaluate with one QC per day (alternating levels between days) and a 1:3.5 s rule.
- 4σ – 6σ (suited for purpose)—evaluate with two levels of QC per day and the 1:2.5 seconds rule.
- 3σ – 4σ (poor performers)—use a combination of rules with two levels of QC twice per day.
- $<3\sigma$ (problems)—maximum QC, three levels, three times a day. Through sigma metrics, it is easy to identify high-risk and low-risk test methods.³

To design the right statistical QC procedure, Westgard suggested the use of Westgard sigma rules as shown in Figure 1.⁴

In resource-poor settings, it becomes imperative to implement measures that avoid wastage while maintaining the desired level

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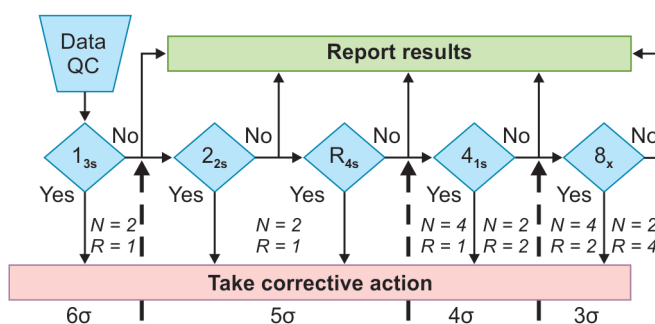


Fig. 1: Westgard sigma rules for 2 levels of control materials

of quality. Hence, practising the use sigma metric to design QC can prove useful in our setting.

In this study, the analytical performance of the following tests done in clinical biochemistry laboratory was evaluated using six sigma methodology: alpha feto protein (AFP), ALP, anti-TPO, calcium, chloride, CK-MB, creatinine, ferritin, folate, free T4, Gamma-glutamyl transferase (GGT), magnesium, potassium, PSA, sodium, total bilirubin, troponin I, TSH, vitamin B12, and for analytes having sigma metric of <3 were compared before and after the application of suggested rules.

MATERIALS AND METHODS

The study was performed at Clinical Biochemistry Laboratory of St. John's Medical College Hospital, Bengaluru, India. Data were collected over a period of 9 months from January to September 2016 from Unity Real Time (URT) software, Bio-Rad Laboratories, Inc. IQC data of all levels that were accepted for analytical run in the laboratory from January to September 2016 were included in the study. Any data points that have been rejected or excluded by the laboratory due to being flagged with errors during QC run, gross mistakes while preparing QC (pipetting errors) that were not accepted for analytical runs, QC runs just before or at the time of equipment breakdown, and multirule criteria applied for QC run were excluded from the study. Total 19 analytes were chosen for the study.

Table 1 shows the method of analysis and the instruments used to analyze the respective analyte.

Sigma metric was calculated for all the analytes for the period of January–June 2016. The analytes which showed sigma metric of <3 were further analyzed during the period of July–September 2016 after the application of rules as suggested by URT.

The study was approved by Institutional Ethical Committee.

Method of Calculating Sigma Metric

The sigma metrics for the various analytes were calculated by the following formula:

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

where TEa is the total allowable error and CV is the coefficient of variation.

TEa values of various parameters were taken from the biological variation database by Dr Carmen Ricos et al. available at www.westgard.com.⁵

CV was determined from the calculated laboratory mean and calculated standard deviation procured from the IQC data.

$$\text{CV } (\%) = (\text{Standard deviation} / \text{Laboratory mean}) \times 100$$

Peer mean was obtained from URT software and bias was calculated by using the formula

$$\text{Bias } (\%) = (\text{Our mean} - \text{Peer mean} / \text{Peer mean}) \times 100$$

Statistical Software

The data were entered and analyzed on Microsoft Excel Version 2016.

RESULTS

The units, QC levels, lab mean, standard deviation (SD), CV, peer mean, and bias for the analytes for the period of January–June 2016 are shown in Table 2.

Table 1: List of analytes, instrument used, and the method of analysis

Analyte	Instrument	Method
AFP	Siemens ADVIA Centaur XP	Chemiluminescence
ALP	Siemens Dimension EXL	Modified Bower's and McComb method/modified Rej
Anti-TPO	Siemens ADVIA Centaur XP	Chemiluminescence
Calcium	Siemens Dimension EXL	Modified o-cresolphthalein complexone
Chloride	Siemens Dimension EXL	ISE, indirect
CK-MB	Siemens ADVIA Centaur XP	Chemiluminescence
Creatinine	Siemens Dimension EXL	Alkaline picrate-kinetic
Ferritin	Siemens ADVIA Centaur XP	Chemiluminescence
Folate	Siemens ADVIA Centaur XP	Chemiluminescence
Free T4	Siemens ADVIA Centaur XP	Chemiluminescence
GGT	Siemens Dimension EXL	G-glutamyl-carboxy-nitroanilide
Magnesium	Siemens Dimension EXL	Methylthymol blue
Potassium	Siemens Dimension EXL	ISE, indirect
PSA	Roche cobas 6000	Electrochemiluminescence
Sodium	Siemens Dimension EXL	ISE, indirect
Total bilirubin	Siemens Dimension EXL	Modified Jendrassik and Grof method.
Troponin I	Siemens ADVIA Centaur XP	Chemiluminescence
TSH	Siemens ADVIA Centaur XP	Chemiluminescence
Vitamin B12	Siemens ADVIA Centaur XP	Chemiluminescence

Sigma metric was calculated for the analytes for the period of January–June 2016 and is presented in Table 3 along with the TEa targets for the analytes.

Analytes having sigma metric of <3 were analyzed in the period of July–September 2016 by applying the suggested rules from URT.

Table 4 lists the analytes that were chosen for further analysis, and existing QC rejection rules and rejection rules suggested by URT software are also shown.

Among 19 analytes performed, 8 showed improvement in sigma value after the application of suggested rules. Table 5 depicts the analytes and levels that showed an improvement in sigma value after the application of suggested rules.

AFP Level 1 (Figs 2 and 3) showed sigma metric of ≥ 3 after the application of suggested rules.

The following analytes also showed an improvement in sigma metric but showed sigma performance ≤ 3 even after the application of suggested rules: calcium L2, chloride L1 and L2, magnesium L1 and L2, sodium L1 and L2.

Method decision charts for calcium L1 and L2 are presented in Figures 4 to 7. We can see from the figures that calcium has not shown much improvement on the application of suggested rules. We can also note that the TEa target is very narrow.

Table 6 shows the list of analytes that did not show any improvement after the application of suggested rules.

DISCUSSION

It is generally believed that if a laboratory follows the regulatory requirements, its performance is good. However, this is not always true.⁶

Table 2: Lab mean, SD, CV, peer mean, and bias for the period of January–June 2016 (*N* = number of data points)

Analyte	Unit	Level	Lab mean (<i>N</i>)	SD	CV	Peer mean (<i>N</i>)	Bias
AFP	ng/mL	1	10.63 (41)	0.61	5.72	11.51 (6,081)	−7.61
		2	91.48 (26)	3.39	3.7	93.16 (4,879)	−1.8
ALP	U/L	1	100.96 (424)	2.64	2.61	98.58 (7,453)	2.42
		2	399.81 (443)	8.44	2.11	397.68 (7,493)	0.54
Anti-TPO	U/mL	1	107.07 (119)	9.36	8.74	113.04 (696)	−5.29
		2	260.79 (38)	12.25	4.7	274.34 (316)	−4.94
Calcium	mg/dL	1	8.800 (222)	0.16	1.81	8.808 (9,716)	−0.01
		2	11.71 (242)	0.24	2.08	11.74 (9,424)	−0.27
Chloride	mEq/L	1	114.90 (440)	2.23	1.94	115.71 (5,593)	−0.7
		2	97.02 (443)	1.89	1.95	97.68 (5,562)	−0.67
CK-MB	ng/mL	1	3.95 (70)	0.19	4.92	3.68 (3,456)	7.4
		2	13.67 (91)	0.65	4.75	12.94 (4,448)	5.6
Creatinine	mg/dL	1	2.84 (137)	0.07	2.34	2.80 (4,278)	1.31
		2	6.17 (143)	0.14	2.31	6.21 (4,284)	−0.71
Ferritin	ng/mL	1	58.74 (102)	3.19	5.42	58.1 (16,351)	1.09
		2	139.52 (99)	11.42	8.18	141.99 (11,991)	−1.74
		3	381.21 (130)	35.68	9.36	383.07 (15,448)	−0.48
Folate	ng/mL	1	2.37 (41)	0.43	18.09	2.49 (12,957)	−4.82
		2	7.07 (50)	0.9	12.79	7.35 (11,237)	−3.82
		3	10.76 (70)	1.25	11.64	11.48 (11,400)	−6.32
Free T4	ng/dL	1	0.71 (99)	0.07	9.49	0.69 (23,778)	2.82
		2	1.94 (100)	0.13	6.7	1.92 (21,777)	0.84
		3	3.13 (129)	0.26	8.35	3.08 (20,118)	1.58
GGT	U/L	1	67.49 (426)	1.72	2.55	62.35 (152)	8.24
		2	176.36 (433)	2.54	1.44	171.16 (155)	3.04
Magnesium	mg/dL	1	2.04 (210)	0.06	3.16	2.07 (6,083)	−1.42
		2	4.68 (227)	0.12	2.63	4.75 (6,010)	−1.31
Potassium	mEq/L	1	3.78 (445)	0.04	1	3.81 (5,447)	−0.85
		2	6.11 (450)	0.07	1.15	6.15 (5,477)	−0.68
PSA	ng/mL	1	0.180 (36)	0.01	5.24	0.1755 (6,534)	2.52
		2	4.39 (26)	0.09	2.04	3.99 (5,161)	9.8
Sodium	mEq/L	1	139.60 (454)	1.36	0.98	140.67 (5,577)	−0.76
		2	125.63 (447)	1.24	0.98	126.65 (5,562)	−0.81
Total bilirubin	mg/dL	1	0.92 (417)	0.03	3.54	0.93 (4,909)	−1.63
		2	4.36 (434)	0.12	2.79	4.38 (4,904)	−0.41
Troponin I	ng/mL	1	0.10 (64)	0.01	6.84	0.096 (3,484)	3.91
		2	4.13 (93)	0.37	9	5.33 (2,382)	−22.59
TSH	μIU/mL	1	0.36 (154)	0.01	3.55	0.37 (23,291)	−2.53
		2	5.1 (147)	0.18	3.6	5.25 (22,066)	−2.93
		3	29.63 (196)	1.05	3.56	30.55 (19,858)	−3.02
Vitamin B12	pg/mL	1	421.66 (107)	30.21	7.17	410.71 (16,149)	2.66
		2	543.21 (101)	38.98	7.18	529.35 (12,925)	2.62
		3	849.06 (126)	40.86	4.81	848.01 (14,083)	0.12

There is still a wide scope of improvement in the quality of laboratory processes, and there is an utmost need to follow the right strategies for doing so to avoid wastage and delivery of wrong results.

A systematic approach is needed to reliably detect clinically significant analytic errors that are beyond allowable inherent errors. This justifies the main objective of the sigma metrics analysis; first, to detect the errors that are more than allowed and then to minimize the identified defects.^{7–10}

In this study, we have carried out sigma metric analysis of analytical performance. Analysis was straightforward, as the information of CV% and bias was readily available with laboratory QC records.

Unity Real Time software suggests rules for each analyte based on its performance. However, there is a general norm to use same rules for all analytes. This may lead to high false rejection in a process with high sigma and low error detection in process with poor sigma.⁶

Table 3: TEa targets and sigma metric for analytes for the period of January–June 2016

Analyte	Level	TEa	Sigma
AFP	1	21.9	2.5
	2	21.9	5.43
ALP	1	12.04	3.66
	2	12.04	5.43
Anti-TPO	1	46.2	4.68
	2	46.2	8.78
Calcium	1	2.55	1.41
	2	2.55	1.1
Chloride	1	1.5	0.4
	2	1.5	0.41
CK-MB	1	30.06	4.62
	2	30.06	5.16
Creatinine	1	8.87	3.25
	2	8.87	3.55
Ferritin	1	16.9	2.91
	2	16.9	1.85
	3	16.9	1.75
Folate	1	39	1.89
	2	39	2.75
	3	39	2.81
Free T4	1	8.05	0.55
	2	8.05	1.08
	3	8.05	0.78
GGT	1	22.1	5.43
	2	22.1	13.22
Magnesium	1	4.81	1.07
	2	4.81	1.33
Potassium	1	5.61	4.76
	2	5.61	4.29
PSA	1	33.6	5.93
	2	33.6	11.67
Sodium	1	0.73	−0.04
	2	0.73	−0.08
Total bilirubin	1	26.9	7.15
	2	26.9	9.49
Troponin I	1	27.9	3.51
	2	27.9	0.59
TSH	1	23.7	5.96
	2	23.7	5.78
	3	23.7	5.81
Vitamin B12	1	30	3.81
	2	30	3.82
	3	30	6.21

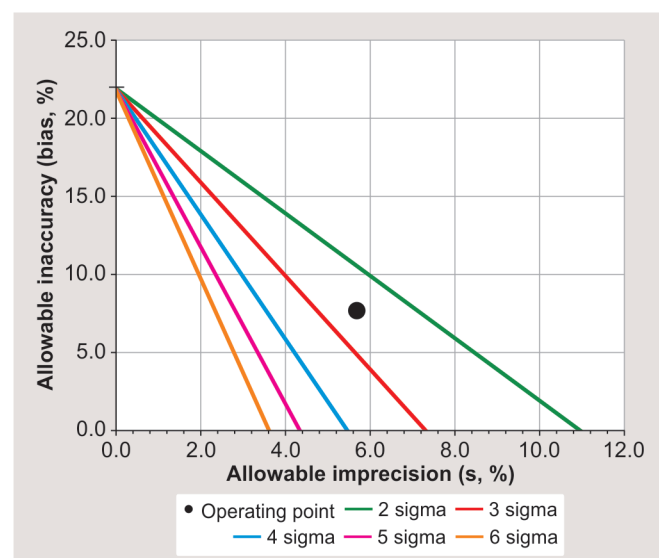
It is suggested that for a process with $\sigma \geq 6$, to avoid false rejections, control limits should be relaxed up to 3.5 SD with N (number of controls to be run per day) = 2; for a five sigma process, 3.0 SD control limits with $N = 2$ have to be used; for a four sigma process, 2.5 SD control limits or a multirule procedure with $N = 4$ have to be used; for a three sigma process, multirule procedures with N of 6 or 8 have to be used. For less than three sigma, method

Table 4: Analytes that showed $\sigma \leq 3$ arranged in ascending order of sigma

Analyte	Level	Sigma	Existing rules	Suggested rules
Sodium	2	−0.08	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Sodium	1	−0.04	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Chloride	1	0.4	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Chloride	2	0.41	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Free T4	1	0.55	13s, 22s, R4s	13s, 22s, R4s, 31s, 8x
Troponin I	2	0.59	13s, 22s, R4s	13s, 22s, R4s
Free T4	3	0.78	13s, 22s, R4s	13s, 22s, R4s
Magnesium	1	1.07	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Free T4	2	1.08	13s, 22s, R4s	13s, 22s, R4s
Calcium	2	1.1	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Magnesium	2	1.33	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Calcium	1	1.41	13s, 22s, R4s	13s, 22s, R4s, 41s, 8x
Ferritin	3	1.75	13s, 22s, R4s	13s, 22s, R4s
Ferritin	2	1.85	13s, 22s, R4s	13s, 22s, R4s
Folate	1	1.89	13s, 22s, R4s	13s, 22s, R4s
AFP	1	2.5	13s, 22s, R4s	12s, 13s, 22s, R4s
Folate	2	2.75	13s, 22s, R4s	13s, 22s, R4s
Folate	3	2.81	13s, 22s, R4s	13s, 22s, R4s

Table 5: Analytes that showed improvement in sigma after the application of suggested rules

Analyte	Level	Sigma before application of suggested rules	Sigma after application of suggested rules
AFP	1	2.5	9.3
Calcium	2	1.1	1.62
Calcium	1	1.41	1.56
Chloride	2	0.41	0.88
Magnesium	1	1.07	2.92
Magnesium	2	1.33	2.75
Sodium	2	−0.08	−0.78
Sodium	1	−0.04	−0.62

**Fig. 2:** Method decision chart for AFP L1 before application of suggested rules

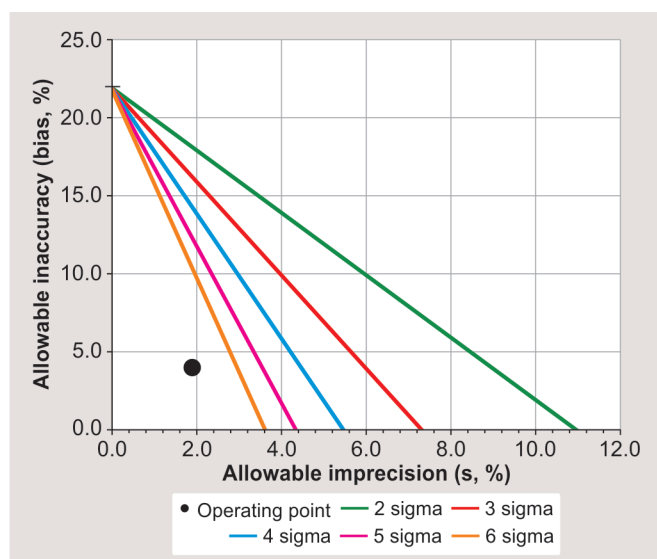


Fig. 3: Method decision chart for AFP L1 after application of suggested rules

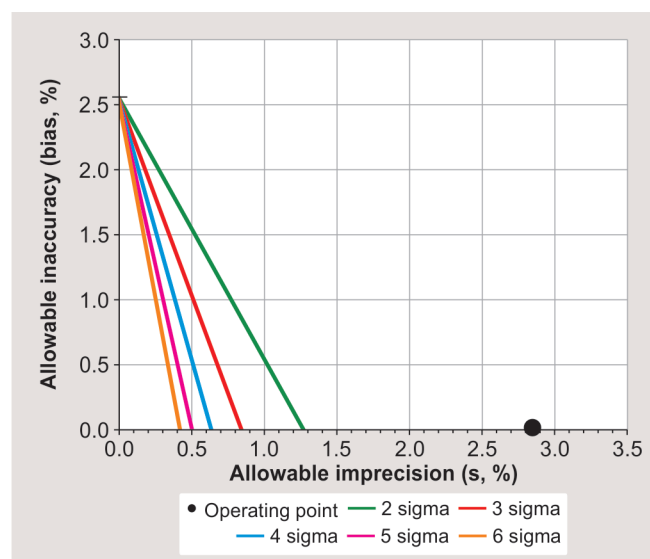


Fig. 4: Method decision chart for calcium L1 before application of suggested rules

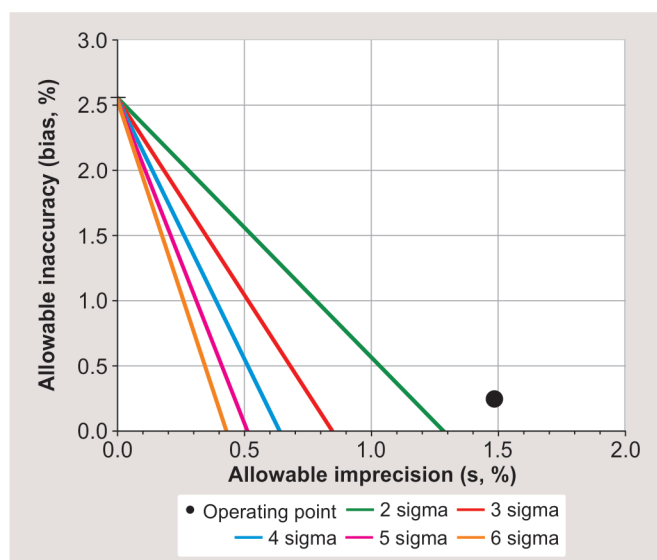


Fig. 5: Method decision chart for calcium L1 after application of suggested rules

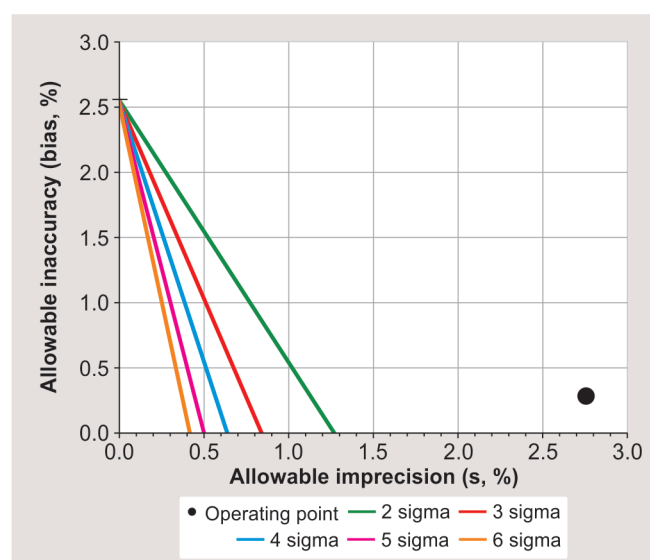


Fig. 6: Method decision chart for calcium L2 before application of suggested rules

performance must be improved before the method can be used for routine diagnostic purposes.^{7,8}

In our study, gamma glutamyl transferase has the highest value of sigma of 13.22 at Level 2 of quality control. Total bilirubin was found to have the highest sigma values at both control levels. It was the only analyte having a sigma value of ≥ 6 for both the levels of QC (7.15 and 9.49 at L1 and L2, respectively). This implies that the analytical method in use is appropriate for detecting both low and high values. The QC strategies that should be implemented in such cases need not be stringent.

Sigma value of ≥ 4 was observed across all control levels for anti-TPO, CK-MB, potassium, PSA, TSH. According to Westgard sigma rules TM, these analytes are suited for purpose.⁴

We have obtained a sigma value of ≤ 3 for all levels of calcium, chloride, ferritin, folate, Free T4, magnesium, and sodium. Level 1 of AFP and level 2 of troponin I also had a sigma value of ≤ 3 . Among

these, level 1 of AFP showed a significant improvement in sigma after the application of suggested rules (2.5 to 9.3).

Root cause analysis should be performed for analytes less than three sigma, which did not improve on the application of suggested rules. Selection of appropriate QC procedures for detecting errors can improve test methods to a certain extent. However, it would be a good practice to choose a test method with a six sigma performance to avoid wastage in repeating tests and troubleshooting and to reduce the cost of quality control. Laboratories must also try to control precision through proper training, instrument maintenance, etc.^{6,11}

Westgard and Burnett published a paper in 1990 on "Precision requirements for cost-effective operation of analytical process." In this paper, they recommended that laboratories should require four sigma performance for any new method and that even more demanding criteria—five sigma to six sigma—may be necessary

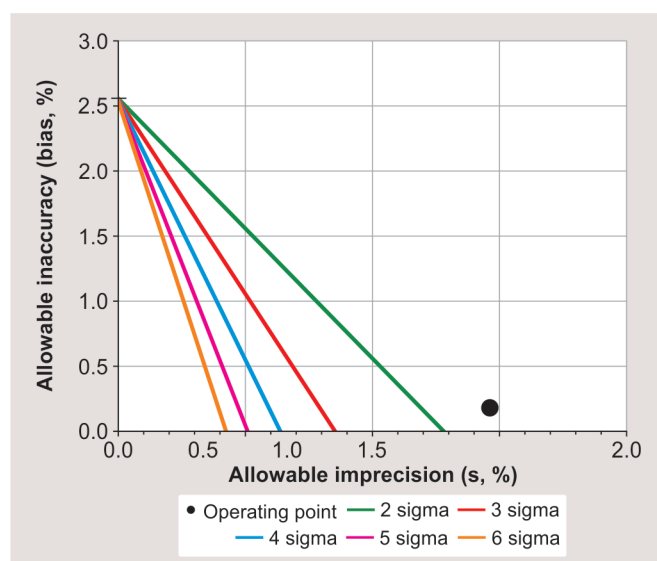


Fig. 7: Method decision chart for calcium L2 after application of suggested rules

for applications where personnel have little training in analytical techniques.^{6,12}

The sigma metrics reported here is a snapshot of the analytical performance at the time the data were collected. The performance however can change over time for several reasons (e.g., reagent lot to lot variation).

Sigma metrics need to be monitored periodically to determine if assay quality has been maintained or not.

CONCLUSION

Quality goals for the laboratories must be decided appropriately, keeping in mind the inherent random errors and performance capability of analyzers. Sigma metrics is an efficient way to control quality by matching the QC rules to the analytical quality of individual assay which leads to the judicious use of QC.

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Table 6: Analytes for which sigma did not improve after the application of suggested rules

Analyte	Level	Sigma before application of suggested rules	Sigma after application of suggested rules
Chloride	1	0.4	0.13
Ferritin	3	1.75	1.33
Ferritin	2	1.85	1.79
Ferritin	1	2.91	2.88
Folate	1	1.89	1.84
Folate	2	2.75	2.09
Folate	3	2.81	1.93
Free T4	1	0.55	0.32
Free T4	3	0.78	0.77
Free T4	2	1.08	0.86
Troponin I	2	0.59	–0.58

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