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Application of sigma metrics in haematology laboratory

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Abstract
Objectives: Sigma metrics analysis indicates; the level quality control has achieved and how far a given process deviates from perfection. Though the concept is not new to quality and utility, its implementation in the haematology laboratory is still in its nascent stages. The study aimed to compare the utility of sigma metrics as a comprehensive quality control tool in Haematology laboratory against the current quality tools of Internal Quality Control (IQC) and External quality assurance scheme for haematology analyser.
Methods: In the present study, IQC data was analysed from June 2021 to September 2021. Control material included 3 batches of second party controls (SPC). Sigma were calculated for five key haematological parameters run in Sysmex XN 350. OPSpecs charts were prepared. Sigma metrics were calculated using the formula Sigma = (TEa – Bias) / CV, Bias was taken from IQC kit insert. Quality goal index was also calculated when needed.
Results: Average sigma metrics achieved for selected parameters was, RBC: 8.1, HB: 7.6, HCT 5.6, PLT: 7.0, and WBC: 6.7. The laboratory has achieved excellent to world class performance in all analytes. Sigma QC rules were applied and modifications proposed to the existing internal QC protocols to reduce the number of rejections.
Conclusion: Labs must preferentially use the sigma metrics and sigma QC rules to design its IQC protocols, for individual parameters, rather than follow the 'one fit for all' recommendation. This would help save precious time, effort, unnecessary control runs and improve efficiency with better focus on quality control, where required.

Keywords: Bias, sigma metrics, OP specs chart, quality goal index

Introduction
Quality is defined as conformance to the requirements of the end users [1]. Quality Control aims at immediate detection of an error by monitoring the accuracy and precision of the analytical processes. Implementation of quality control (QC) is a continuous dynamic process to ensure that test results produced by the laboratory are reliable.
Sigma in statistics is used to represent the Standard deviation (SD), which is an indicator of the degree of variation in a set of processes. Sigma measures how far a given process deviates from perfection and it is a popular quality management system tool employed for process improvement. Evaluation of errors in terms of sigma metrics is more meaningful than number of defects alone. For analytical process of laboratory system, sigma metric analysis identifies the errors in quality indicators of the process and provides correction of errors on the basis of results [2].
Traditionally, quality control programme in haematology laboratory involves the use of internal quality controls (IQC) and External quality assurance scheme (EQAS). The IQC uses commercial controls which have known values against which daily runs are plotted on Levey-Jennings (L.J.) Charts and evaluated by the Westgard rules, known to all for decades. Westgard has long advocated customizing Statistical QC procedures, such as "Westgard Rules", to take into account the quality required for the intended use of a test and the imprecision and bias observed for a method. However, this methodology has made with what many calls 'one set of rule, rules all'. The Westgard rules that Lab has applied throughout all the various parameters, these increases number of unnecessary reruns that we have to do when there is even a single parameter go outlier, which not only increases the time but also puts a strain on the already trained staff, equipment and resources especially on resource constraint settings.

It has famously been said by Westgard himself that we need to move to better metrics where we can individualise these rules and follow them according to the standard limits of defects that they are usually pertaining commonly to.

The "Sigma" in Six Sigma refers to the benchmarking scale upon which all process defects are judged. Defects can be counted or estimated and then converted to a defects-per-million (DPM) ratio (Table 1). Sigma metrics is being routinely practiced by many clinical biochemistry laboratories and various studies and literatures are also available for the same, but in clinical haematology laboratory, though the concept is not new to quality and utility, its implementation is still in its nascent stages.

In book Basic Quality Management Systems [5] written by James Westgard in 2014, introduced a new tool that is quicker and easier to use than previous tools. They called this tool "Westgard Sigma RulesTM" to distinguish this approach from the original Westgard Rules. Sigma metrics with that respect provides us individuality where it encompasses the IQC and EQAS for individual parameter and then based on the sigma metrics value we may be able to decrease the number of re runs by choosing the right set of sigma rules which is shown below.

Sigma rule for the three level of controls are,

- 6-sigma quality requires only a 1_{3s} rule and 1 measurement on each of 3 levels of controls.
- 5-sigma quality requires adding the 2 of 3_{2s} and R_{4s} rules for use with 1 measurement on each of 3 levels of controls.
- 4-sigma quality requires adding a 3_{1s} rule for use with 1 measurement on each of 3 controls.
- < 4-sigma quality requires a multirule procedure that includes the 6_x rule and a doubling of control measurements to a total of 6, which suggests that the 3 levels of controls be analysed in duplicate in one run (N=6, R=1) or the day's work be divided into 2 runs with 3 control measurements per run (N=3, R=2). If a 9_x rule were substituted for the 6_x rule, then a day's work could be divided into 3 runs with 3 controls per run (N=3, R=3).[6]

Sysmex XN 350 is an automated haematology analyser that enables quantitative identification, and existence ratio analysis of tangible components of blood and body fluid (red blood cells and its indices, white blood cells and platelets) by means of electrical impedance, laser light scattering and fluorescent labelling.

To attain the aim, following were the objectives to be achieved:

1. Assess the performance of selected haematological parameters on a Sigma scale by calculating the sigma metrics
2. Use the sigma values to plot OPSpecs chart(s) for all the parameters to redesign the internal quality control (IQC) plan for haematology parameters, if necessary.

Material and Method

Study design: The present observational study was carried out at Central Diagnostic Laboratory of Shree Krishna Hospital, Karamsad from June 2021 to September 2021. Commercial [Sysmex provided] controls were run on the Sysmex XN-350 automated haematology analyser.

Methodology: According to internal quality control policy of the laboratory, two levels of controls amongst Low (L1), Normal (L2) or High (L3) of control material were being used in each run on 8 hourly bases, based on the recommendations provided by National Accreditation Board for Testing and Calibration Laboratories (NABL) [7]. Total allowable error (TEa) values of various parameters were taken from the Clinical Laboratories Improvement Act (CLIA) guidelines [8]. Quality goal index (QGI) ratio was calculated to assess the problem whenever acceptable quality was not achieved for any level of IQC to assess whether it was due to imprecision (IQC/ CV%) or due to inaccuracy (Bias%) of which formula is mentioned below.

- For each level of control, coefficient of variation (CV) was calculated from mean and SD of IQC data with minimum 20 runs, and all calculations done as per given equation:

Equation 1: CV% = SD / mean x 100

- Bias was calculated from Peer review provided in control kit insert,

Equation 2: Bias = [(our laboratory mean–target mean)/target mean] ×100

- Sigma metrics for the various analytes was calculated by the following equation:

Equation 3: Sigma (σ) = (TEa-Bias%) / CV%

Where
TEa =total allowable error,
CV=coefficient of variation

- The QGI ratio was calculated using the following formula:

Equation 4: QGI = Bias/1.5 × CV%

Results and Discussion

IQC data from 08 control run cycles (with a minimum of 20 runs) were collected from the Sysmex XN 350 automated haematology analyser. Mean, SD, and CV of each run were calculated and were as shown in Table 2. TEa, Calculated Bias and Sigma-value are available in Table 3.

CV% is a measure of imprecision. Calculation of CV% is based on control material test results data for the IQC process. Mean and SD of the daily controls with assigned values is calculated and CV% is calculated by the formula mentioned above. Clinical and Laboratory Standards Institute (CLSI) recommends that the data be obtained at least by running 3-6 months routine IQC test. Control test results were performed twice daily for twenty days of these; there were two examination runs within a single day, each run consisted of 10-20 replicates control material to assess within-day or between-run imprecision. A single run with at least 20 replicates of control material assesses within-run imprecision or repeatability [9].

Bias is the difference between the measured result and actual value. It is used to describe inaccuracy of the method. Lower the bias more is the accuracy. There are many methods to calculate bias. Data of bias can be obtained from reference material or the reference method; the mean of a peer group; the mean of proficiency testing or external quality assessment survey; or a comparative method [10]. In the present study, bias was obtained as the mean of a peer group available in the kit insert of control material and

sigma was calculated, which is comparable with other studies.

Total allowable error (TEa) is essentially an analytical quality goal that specifies the maximum amount of error-both imprecision and bias combined—that is allowed for an assay, and ensures clinical usefulness of the single test result. If the difference between the true concentration of an analyte and the reported concentration in a patient's specimen exceeds TEa, the result is considered to be unreliable. Selecting TEa is based on clinical outcomes or the biological variation of the analyte first, followed by a state-of-the-art approach if the first two models were unavailable Goals set by regulatory bodies (CLIA'88 in the United States) and Proficiency Testing/EQA organizers are easily available and understood. Hence, in the present study too, the TEa is applied as per CLIA guidelines.

The highest Sigma-value was noted in platelets with high level controls (13.5). Haematocrit at normal level controls had the lowest Sigma-value (2.6). Out of 40 occasions, more than six sigma was achieved in 26 occasions (26/40) (65%) while less than 3 sigma was observed on (05/40) (12.5%). Average Sigma-value for different parameters are as follows, RBC: 8.1, HB: 7.6, HCT: 5.6, PLT: 7.0, and WBC: 6.7. QGI ratio was calculated to assess the problem whenever acceptable quality was not achieved for any level of internal quality control, to assess whether it was due to imprecision (IQC/ CV%) or due to inaccuracy (Bias%). For analytes which fall short of three Sigma quality, a QGI score of < 0.8 indicates imprecision, QGI > 1.2 indicates inaccuracy, and QGI score 0.8-1.2 indicates both imprecision and inaccuracy. Causes for the same are depicted in Table 5. Unacceptable sigma was noted in HCT (one occasion), PLT (Three occasions) and WBC (One occasion). On these instances, problem of imprecision was discovered for HCT and PLT while WBC had problems of both imprecision and inaccuracy.

Normalised OPSpecs chart were prepared to create the QC guidelines that need to be followed for the various parameters. We chose N=3, 50% analytical quality assurance (AQA) chart as the lab runs two level of controls two times a day; hence, if the error was missed in the first run, the same could still be reflected in the other run. Value of X and Y axis were calculated from normalised OPSpecs calculator which is available on Westgard website [12]. The charts were downloaded from the website too, and the final values plotted on the chart manually. Subsequently, control rule(s) were selected whose operating limits are above our normalized operating point. The key on the right side of the chart was used to identify the control rule (s).

Recommended Sigma rejection rules of Sysmex XN 350 based on Current Sigma

For all parameters, based on the OPSpecs chart and sigma rules, the 1_{2s} and 2_{2s} rejection may be accepted, meaning that these rejection rules may not be applied and work can be continued without having to run the quality control material again. When we are running level 3 control, we need to apply 1_{3s} only in all the parameters, other all rules can be rejected. When the number of rejections is reduced, the number of re-runs of controls is reduced and the laboratory's working is smoother as precious time is saved.

Fuadi, Robiul *et al*. conducted a study in Department of Clinical Pathology Airlangga University/ Soetomo Hospital Surabaya, Indonesia [13]. They analysed the data from the routine Complete Blood Count (CBC) test results of assayed control material in July-August 2016. Examination of the material was performed once daily with Abbot Cell Dyne Ruby haematology analyser. Data from the mean of internal quality control material results and the target value of control material was used to calculate the bias. The highest Sigma-value was achieved by platelet in normal level (8.7). HCT at normal level control gave the lowest Sigma-value (1.7). Parameters with Sigma-value of more than six were achieved by Haemoglobin for normal level control (6.7), WBC count at low, normal and high-level controls (7.1, 8.7 and 6.4 respectively). Sigma-value of less than three was obtained for Haemoglobin for high level control (2.6), platelet count for low level control (2.5), HCT for normal level control and high-level controls (1.7 and 2.3 respectively). Average Sigma-value for Haemoglobin was 4.3, HCT was 2.5, RBC count was 3.6, WBC count was 7.3, and PLT count was 6.4. To design internal quality control procedure by Sigma-value they plotted normalized OP Specs chart for N=3, 50% AQA and concluded that HCT Gave the worst performance. They proposed to improve it by increasing the number of QC runs.

Conclusion

The comparison of sigma metrics analysis with IQC and EQAS showed that the six-sigma methodology assesses a combination of imprecision and inaccuracy together, which takes the quality assessment of the parameters a step further. Based on sigma metrics, Sysmex XN 350 has given good sigma values for all the parameters and appears to be giving excellent performance. Amongst the Haematology parameters, world class sigma performance was obtained on Sysmex XN 350 in RBC: L1 and L3, HB: L1, L2 L3, HCT: L1 and L3, Platelet count: L3 and WBC: L2 and L3.

Since the same internal control material is used for obtaining values of different parameters, it would be possible to reduce the number of runs only if the same sigma levels are obtained for all the haematology parameters as in the case of biochemistry parameters where different control materials is used for different parameters. However, it is possible to apply the Sigma rules and reduce the number of rejections for individual control for individual parameters. When the number of rejections are reduced, the number of reruns of controls is reduced and the laboratory's working is smoother as precious time is saved as not having to halt work and work up on QC rejections. QGI ratio helped in giving direction to the root cause analysis whenever sigma metric was below the acceptable limit (<3). OPSpecs charts can be considered to be the report card of the laboratory, since the QC performance of all the parameters of an equipment, for all levels of controls, can be seen in the same chart. There is of course, the added advantage of recommending the QC procedures (rejection rules) that need to be followed on the basis of observed imprecision and inaccuracy.

Haematology Labs must preferentially use the sigma metrics and sigma QC rules to design its internal QC protocols, for individual parameters rather than follow the one fit for all recommendations by different bodies. While it may not be possible to reduce the number of runs of the internal controls, it is possible to redesign the QC rejections for individual parameters for the controls that are used. This would help save precious time, effort, unnecessary runs and improve efficiency with better focus on quality control, where required.

Conflict of interest: None declared.

Table 1: Sigma performance table [3, 4]

Sigma value	Reference Level	Defect per million opportunities
6	World class	3.4
5	Excellent	233
4	Good	6210
3	Marginal	66,807
2	Poor	308,537
1	Unacceptable	690,000

Table 2: Mean, SD and CV% of haematological parameters

Parameters	RBC			HB			HCT			PLT			WBC		
Lot no.	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
11271401 (L1)	2.3	0.0	1.0	6.0	0.1	1.5	17.5	0.2	1.2	61.0	5.5	9.0	2.6	0.1	3.5
11271402 (L2)	4.3	0.1	1.5	12.3	0.2	1.6	34.6	0.7	1.9	225	8.3	3.7	7.2	0.2	2.7
11271403 (L3)	5.2	0.0	0.8	16.4	0.2	1.0	45.0	0.5	1.0	487	10.6	2.2	16.4	0.4	2.2
11601101 (L1)	2.3	0.0	1.1	5.4	0.1	1.5	16.2	0.2	1.1	92	7.2	7.9	3	0.1	2.2
11611102 (L2)	4.3	0.1	1.4	11.9	0.1	1.2	34.5	0.5	1.5	247	15.3	6.2	7.1	0.1	1.9
11581103 (L3)	5.0	0.0	0.7	15.7	0.1	0.9	44.8	0.4	0.8	540	12.7	2.3	16.5	0.2	1.5
12111402 (L2)	4.3	0.1	1.4	12.9	0.1	0.8	36.6	1.0	2.6	258	16.4	6.4	7.3	0.1	1.9
12111403 (L3)	5.3	0.0	0.6	16.5	0.1	0.7	46.4	0.6	1.2	610	10.7	1.7	16.9	0.3	1.5

Table 3: TEa, Bias, Sigma metrics and QGI ratio of haematological parameters

Parameters	RBC			HB			HCT			PLT			WBC		
Lot no.	TEa	Bias	Sigma [QGI]*	TEa	Bias	Sigma [QGI]*	TEa	Bias	Sigma [QGI]*	TEa	Bias	Sigma [QGI]*	TEa	Bias	Sigma [QGI]*
11271401 (L1)	6%	-2.6	8.6	7%	-3.2	6.2	6%	-2.2	6.9	25%	-15.3	4.5	15%	4.9	2.9 [0.9]
11271402 (L2)	6%	-2	5.4	7%	-1.6	4.8	6%	-2	4.2	25%	-1.3	7.1	15%	3.3	4.3
11271403 (L3)	6%	-1.9	9.9	7%	-1.2	7.2	6%	-1.7	7.7	25%	-4.7	13.5	15%	2.8	5.6
11601101 (L1)	6%	-2.6	7.8	7%	-3.6	6.4	6%	-1.2	6.6	25%	2.2	2.9 [0.2]	15%	-3.2	8.3
11611102 (L2)	6%	-2.5	6.1	7%	-1.7	6.4	6%	-0.9	4.6	25%	7.9	2.8 [0.8]	15%	-0.1	8.0
11581103 (L3)	6%	-2	11.4	7%	-1.9	8.8	6%	-0.7	8.3	25%	-2.9	12.1	15%	0.9	9.4
12111402 (L2)	6%	-1.4	5.3	7%	-2.3	10.3	6%	-0.8	2.6 [0.2]	25%	7.9	2.7 [0.8]	15%	3.3	6.2
12111403 (L3)	6%	-0.2	10.3	7%	-1.8	11.1	6%	1.3	3.9	25%	7.4	10.4	15%	2.1	8.6
Average sigma			8.1			7.6			5.6			7.0			6.7

* - QGI ratio is calculated when sigma was unacceptable (<3), mentioned in the squared brackets.

Table 4: Causes for imprecision and inaccuracy in the laboratory [11]

Inaccuracy	Imprecision
Intrinsic method bias	Matrix interference
Instrument bias	Mechanical variation
Reagent lot bias	Electrical interference
Calibration bias	Photometer/detector variation
Within run bias	Specimen problem (fibrin clots)

Table 5: Current and recommended Sigma rejection rules of Sysmex XN 350 based on Current Sigma

Parameter	Current Rejection rule applied by the lab based on NABL 112 for all levels(L1, L2, L3)	Current Sigma			Recommended Sigma rejection rule based on sigma rule and OP Specs chart		
		L1	L2	L3	L1	L2	L3
RBC count	1_{3s}, 2_{2s}, R_{4s}, 4_{1s} and 10x	8.2	5.6	10.5	1_{3s}	1_{3s},2of 3_{2s} and R_{4s},	1_{3s}
Haemoglobin	1_{3s}, 2_{2s}, R_{4s}, 4_{1s} and 10x	6.3	7.2	9	1_{3s}	1_{3s}	1_{3s}
HCT	1_{3s}, 2_{2s}, R_{4s}, 4_{1s} and 10x	6.6	3.8	6.6	1_{3s}	1_{3s} ,2of 3_{2s} and R_{4s}, 3_{1s}, 6x	1_{3s}
Platelet count	1_{3s}, 2_{2s}, R_{4s}, 4_{1s} and 10x	3.7	4.2	12	1_{3s} ,2of 3_{2s} and R_{4s}, 3_{1s} ,6x	1_{3s},2of 3_{2s} and R_{4s}, 3_{1s}	1_{3s}
WBC count	1_{3s}, 2_{2s}, R_{4s}, 4_{1s} and 10x	4.6	6.1	7.8	1_{3s} ,2of 3_{2s} and R_{4s}, 3_{1s}	1_{3s}	1_{3s}

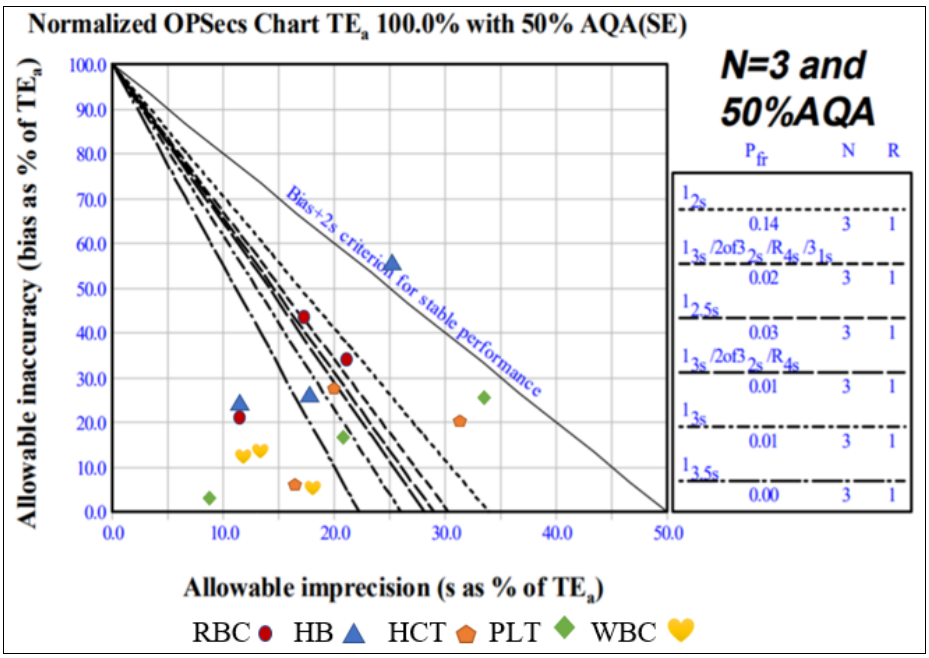

Fig 1: Normalised OPSpecs chart

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