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Research Article

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Setting up an own laboratory performance-based internal quality control plan - a model for complete blood count

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Abstract

Quality Control (QC) in Romania is regulated by the Order of the Minister of Health no. 1608/2022 that modifies the previous Order 1301/2007. The new version of the Order introduces a more scientific approach by requesting the laboratories to assess test performance and then elaborate an appropriate internal QC plan. The aim of this study was to demonstrate how to design a QC plan for complete blood count (CBC) in an Emergency Laboratory with continuous activity, in order to comply with the new Order 1608/2022. QC data obtained over a three-month period (April-June 2022) from the Sysmex XN-1000 instrument of the Emergency Laboratory of the County Emergency Clinical Hospital of Târgu Mureș were included. In order to establish an appropriate QC plan, two models were applied and the following parameters were calculated: the number of daily QC runs (N), the probability of false rejection (Pfr), the QC frequency (run size), and the required QC rules. White blood cells achieved high performance, while Hematocrit performance was poor. Different levels of performance were achieved for Platelets. We emphasize that, when all parameters are measured on the same instrument, QC frequency and Pfr should be adjusted in order to develop a QC plan that "fits" all the parameters of the CBC as a whole. In our Emergency Laboratory, the calculated QC plan for CBC is N=2, Pfr=0.03, multi-rule 1:3s/2:2s/R:4s, and a run size of 95 samples which is approximately the same as the number of CBCs performed during one 12-hour shift.

Keywords: *cytology, quality control and evidence based laboratory medicine*

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Introduction

Statistical Quality Control (SQC) in Romania is regulated by the Order of the Ministry of Health no. 1608/2022 (1) that modifies article 23 of the previous Order 1301/2007 (2). In the previous version of the Order, a QC run was required every 8 hours (2), regardless of the method or its performance. The new version of the Order introduces a more scientific approach by requesting the laboratories to assess test performance and then elaborate an appropriate internal quality control plan (IQC).

Performance of a test may be assessed against known and widely accepted performance requirements, but what are the requirements suitable for the laboratory? Total Allowable Error (TEa) recommended by different authorities in

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the field (CLIA – Clinical Laboratory Improvement Amendments, EFLM – European Federation of Clinical Chemistry and Laboratory Medicine) can vary significantly. For instance, TEa for Platelets (PLT) is 25.0% as per CLIA (3) and 13.5% according to EFLM (4). Another factor that will greatly influence the TE and subsequently the Sigma value is the medical decision level (MDL) at which the TE is reported. According to the new Order (1) and the ISO 15189:2013 Standard (5), at least one normal and one abnormal QC must be run daily by laboratories. In some cases, the assigned values of the commercial QC materials are not similar to the MDLs (6).

The statistical analysis and details about run sizes are described in the Clinical & Laboratory Standards Institute (CLSI) C:24 guidelines (7). The performance of a test may be evaluated by using Six Sigma, which combines the method's imprecision, TEa, and Bias in a single equation. Once the Sigma score is calculated, a set of appropriate rules for interpreting the SQC may be defined (8). The higher the Sigma score, the lesser control events and SQC rules are needed for lower patient risk. The frequency of control events may be calculated by including in the equation the Sigma score, the probability of false rejection (P_{fr}), and a patient risk value that is acceptable for the laboratory (9). Thus, in order to comply with the new Order, each laboratory can establish their own SQC strategy based on analytical performance, working hours, and other particularities. The aim of this study is to demonstrate how to design a QC plan for complete blood count (CBC) in an Emergency Laboratory with continuous activity, in order to comply with the new Order 1608/2022.

Material and method

In the Emergency Laboratory of the County Emergency Clinical Hospital of Târgu Mureș, Romania, patient samples are continuously received and tested. Over a 24-hour period, about 250-300 CBCs are performed by the laboratory on a Sysmex XN-1000 instrument (Sysmex Corporation, Japan) and, according to the previous Order, three levels of commercial control (low, normal, high) are performed every 8 hours. For this study, all QC data obtained over a 3-month period (April-June 2022) were included. Own laboratory means and standard deviations (SD) computed by the instrument's software were used. Only CBC parameters directly measured by the instrument were considered in this study: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), and platelets (PLT). The QC frequency calculator available on-line (10) was used and two models were applied (A and B). Bias was set to 0 since both models apply to a single instrument. Imprecision was derived from the QC SD and CLIA TEa was used for calculations. The two models use different critical decision levels: model A uses MDLs from the guidelines, while model B uses the mean value of each QC level. Details about each model are shown in Table 1.

Table 1. Data used for models A (based on MDLs from the guidelines) and B (based on the mean value of each QC level).

Data	Model A	Model B
Critical decision level	Medical decision level (MDL)	QC mean
TEa	CLIA regulation	CLIA regulation
Bias	0	0
Imprecision	QC SD	QC SD

Abbreviations: CLIA – Clinical Laboratory Improvement Amendments, QC – quality control, SD – standard deviation, TEa – total allowable error., MDL - medical decision level

Results

Table 2 shows the Sigma scores for each CBC parameter, computed for both models, along with the data used for calculation. The critical decision levels and imprecision are reported in measurement units, not in percentage values.

Run sizes for each control level, QC rules, and the number of QC replicates required for minimal patient risk are shown in Table 3.

For clarification, we will use RBC as an example (Table 3): with current performance and a desired daily QC frequency of N=2, the maximum run size for each of the 3 control levels is 1000, 346, and 1000 samples for model A and 95, 1000, and 1000 samples for model B. In this

Table 2. Data used for calculating Sigma scores for both models A (based on MDL) and B (based on QC mean). Critical decision levels (A - MDL, B - QC mean) and imprecision (SD) are reported in measurement units, not in percentage values. In order to better associate QC levels with similar MDLs, QC levels low (L), normal (N), and high (H) are sometimes presented in a different order in the table. QC levels that do not coincide at least remotely with any of the MDLs, are reported on separate lines of the table.

	TEa (%)	MDL	TEa × MDL	SD	QC level	QC mean	TEa × QC mean	SixS Model A	SixS Model B
WBC (10^3/µL)	15	0.5	0.08	0					
	15	3	0.45	0.08	L	2.97	0.45	5.63	5.63
	15	12	1.80	0.2	H	16.57	2.49	9	12.42
	15	30	4.50	0.2				22.5	5.1
	15			0.12	N	6.81	1.02		
RBC (10^6/µL)	6			0.03	L	2.34	0.14	7	4.67
	6	3.5	0.21	0.04	N	4.3	0.26	5.25	6.5
	6	5.5	0.33	0.04	H	4.96	0.30	8.25	7.5
HGB (g/dL)	7	4.5	0.32	0.07	L	5.2	0.36	4.57	5.14
	7	10.5	0.74	0.09	N	10.7	0.75	8.22	8.33
	7	17	1.19	0.09	H	15.5	1.09	13.22	12.11
	7	23	1.61						
HCT (%)	6	14	0.84	0.27	L	15.8	0.95	3.11	3.52
	6	33	1.98	0.43	N	32.1	1.93	4.6	4.49
	6	56	3.36	0.51	H	44.5	2.67	6	5.24
	6	70	4.20					7.12	
PLT (10^3/µL)	25	10	2.50					0.51	
	25	50	12.50					2.55	
	25	100	25.00	4.9	L	97	24.25	5.1	4.95
	25	600	150.00	11.6	H	557	139.25	12.93	12
	25	1000	250.00					21.55	
	25			9.6	N	260	65.00		6.77

Abbreviations: HCT – hematocrit, HGB – hemoglobin, H – high (control level), L – low (control level), MDL – medical decision level, N – normal (control level), PLT – platelets, QC – quality control, RBC – red blood cells, SixS – Six Sigma score, TEa – total allowable error, WBC – white blood cells.

Table 3. QC events and SQC rules that may be applied based on analytical performance. *in this scenario, N=2 because our laboratory has a 12-hour work shifts schedule and a QC is desired at the beginning of each work shift. **maximum run size for each of the three control levels.

Parameter	N* / Pfr	Run Size** (Model A)	Run Size** (Model B)	Required QC Rules
WBC	N=2, Pfr =0.0	797/1000/1000	797/248/1000	1:3s
RBC	N=2, Pfr =0.01	1000/346/1000	95/1000/1000	1:3s/2:2s/R:4s
HGB	N=2, Pfr =0.01	158/1000/1000	669/1000/1000	1:3s/2:2s/R:4s
PLT	N=2, Pfr =0.01	410/1000/1000	603/1000/1000	1:3s/2:2s/R:4s
HCT	N=2, Pfr =0.03	4/71/1000	34/557/1000	1:3s/2:2s/R:4s

Abbreviations: HCT – hematocrit, HGB – hemoglobin, N – daily QC frequency, Pfr – probability of false rejection, PLT – platelets, QC – quality control, RBC – red blood cells, WBC – white blood cells.

case, the required QC rules are 1:3s, 2:2s, and R:4s. Any violation of these rules is caused by an error in 99% of cases (P_{fr}=0.01).

Discussions

For WBC, there are four MDLs in the literature, but only three levels of commercial QC are available. Only one QC level (low; mean 2.97 × 10^3/µL) is close to a MDL (3.0 × 10^3/µL). The TEa for this MDL is 0.45 × 10^3/µL and a SD of 0.08 × 10^3/µL was obtained in the investigated laboratory. A Sigma score of 5.63 was calculated both for model A and model B. In a study that compared Six Sigma performances of CBC parameters when different values of TEa were used, a Sigma of 1.74 was obtained for WBC (11). For the low QC level, if only one QC is performed (N=1), a run size of 176 samples is recommended. The next MDL available is 12 × 10^3/µL, while the mean value for the normal QC level is 6.8 × 10^3/µL. Since TEa is 1.02 × 10^3/µL for model A and 1.8 × 10^3/µL for model B, the Sigma score was >6 in both cases, but the two models have different run sizes at N=1: 363 for model A and 64 for model B. For the high QC level, with N=1 and 3:1s as the only QC rule, the calculated run sizes are 363 for both models with a P_{fr} of 0.001. However, since the investigated emergency laboratory has a two-shift schedule, N=2 is better suited. In this case, the maximum run sizes are 248 for the normal QC level and 1000 for the high QC level.

For RBC, there are two MDLs and three levels of control available. The lowest MDL value (3.5 × 10^6/µL) does not coincide with either of the QC levels (low: 2.5 × 10^6/µL; normal: 4.5 × 10^6/µL). With a difference of 0.54 × 10^6/µL, the high QC level is closer to the high MDL (5.5 × 10^6/µL). RBC Sigma scores in this study were >5 for both models at all three levels of control. The Turkish study mentioned above reported an average Sigma score of 3 when CLIA requirements were used (11). The recommended run sizes are 363 for model A, but only 95 for the low QC level of model B if N=1 and only the 1:3s rule is used. In the investigated laboratory, about 250-300 CBCs are performed daily. In this case, for the desired N=2, a multi-rule approach is better suited. With N=2 and P_{fr} of 0.01, the combination of rules 1:3s/2:2s/R:4s would allow run sizes of 95 samples for the low QC level and 1000 samples for the normal and high QC levels.

For HGB, there are four MDLs in the literature and three levels of QC. The highest MDL is 22 g/dL, a value far-off from any QC mean value. In this study, HGB Sigma scores were >4 for both models. These findings are not similar to those reported by another study: Sigma 3.11 (12). For model A, a run size of 76 is recommended for the low QC level, with run sizes of 1000 for the other two levels. For Model B and N=2, a run size of 273 is recommended for the low QC with 1:3s as the only rule. However, with a multi-rule approach (as needed because of RBC performance, see above), the suitable run size of 158 samples is calculated for HGB.

For PLT, there are five MDLs and three levels of control. Sigma scores were >4 for model B and >5 for three out of five MDLs in model A. For the two lowest MDLs, poor PLT Sigma scores were obtained. Sigma values calculated with model A were similar to those reported in other studies on the same instrument. A low Sigma score of 2.5 was reported in a study that used CLIA requirements for the low QC level (12). With model A, run sizes of 603 samples (low) and 1000 samples (normal and high) are recommended if the same combination of rules as mentioned for HGB and RBC is used. With model B, run sizes of 410 samples (low) and 1000 samples (normal and high) were calculated when N=2 and the same combination of rules is applied.

For HCT, the low and normal QC levels are similar to the MDLs. For the low QC level, Sigma scores of 3.11 and 3.52 were obtained in models A and B, respectively. These data are similar to those reported in the literature: Sigma scores <3 were found regardless of the quality requirements used (11). In a study performed on the same instrument, the Sigma score was <3 for the low HCT level (12). Since HCT shows poor performance even with N=2 and a multi-rule approach, the run size is 11 for the low QC level. To ensure an appropriate and practical QC plan for HCT, the low QC level must be run additionally. With N=3 and P_{fr}=0.02, a feasible run size of 34 samples is calculated for the low QC level of HCT.

An earlier study performed in the investigated laboratory on an older Sysmex instrument reported low QC level Sigma scores of 2.31 for PLT and 2.96 for HGB (13). The study used CLIA TEa and QC means as decision levels, similar to model B. Based on the performance at the time of this study, run sizes of 50 samples were acceptable with N=2 for each control level, P_{fr}=0.001, and a multi-rule approach (1:3s/ 2-of-3-2s/ R:4s/ 3:1s).

Conclusion

In this study, high performance was achieved for some CBC parameters such as WBC and poor performance was recorded for others such as HCT. For some parameters, different levels of performance were achieved among control levels: PLT showed low performance at the low control level, but excellent performance at the high control level. We emphasize that, when all parameters are measured on the same instrument, QC frequency and P_{fr} should be adjusted in order to develop a QC plan that “fits” all the parameters of the CBC as a whole. In the investigated laboratory, the calculated QC plan for CBC is N=2, P_{fr}=0.03, multi-rule 1:3s/ 2:2s/ R:4s, and a run size of 95 samples which is approximately the same as the number of CBCs performed during one shift. Thus, in order to ensure adequate error detection, QC will be performed at every shift change on all available control levels, with an additional control for low QC level. This study has general applications in the field of laboratory quality control. However, in particular, this research work comes in response to the paradigm shift brought about by the recent Order of the Romanian Ministry of Health no. 1608/2022. Therefore, here we presented a model for how clinical laboratories in Romania could elaborate their own CBC IQC plan in accordance with the new legislation. Further studies, on investigations other than CBC or based on different statistical models, may be useful in order to establish the best approach to own laboratory performance-based QC planning. We sincerely hope that our research may prove useful to our colleagues and we are looking forward to other laboratory professionals in Romania sharing their experiences and opinions.

Abbreviations

CBC complete blood count
CLIA Clinical Laboratory Improvement Amendments
CLSI Clinical & Laboratory Standards Institute
EFLM European Federation of Clinical Chemistry and Laboratory Medicine
HCT hematocrit
HGB hemoglobin
ISO International Organization for Standardization
IQC internal quality control
MDL medical decision level
P_{fr} probability of false rejection
PLT platelets
QC quality control
RBC red blood cells
SD standard deviation
SQC statistical quality control
TE total error
TEa total allowable error
WBC white blood cells

Authors' contributions

ORO and MD designed the study. ORO, ECP, and IBM performed data acquisition, analysis, and interpretation. All authors participated in drafting the work and revising it critically. All authors agreed to be accountable for all aspects of the work and have read and approved of the final manuscript. ORO and ECP have contributed equally to this work and share first authorship.

Conflict of interest

The authors declare no conflicts of interest.

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