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Research Article

Utilization of Repeat Patient Sample as an Internal Quality Control Measure: The Pilot Study Results

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A B S T R A C T

Introduction: To provide reliable result for every sample, internal and external quality control (QC) measures) must be in place in a clinical laboratory. The present study was designed to asses and re-establishes the utility and efficacy of random patient samples as a regular QC practice.

Method: For quality control (QC) of hematology section a random repeated patient's sample technique was practiced, the difference between present day sample result and that on immediate previous day was calculated. The differences for Hemoglobin, Red Blood Cells Total Leukocyte count and Platelet count were noted. The mean and standard deviation of difference were calculated, with and without considering the plus (+) and minus (-) sign. Levey–Jenning's chart (LJ chart) and Cusum chart of the signed and unsigned differences were plotted for all these four hemogram parameters.

Results: In a total of 246 repeat sample differences, a higher number (49) of Westgard rules violations was observed in LJ. Chart of signed differences, whereas lower number (30) of violations of Westgard rules were observed on analysis of absolute differences. The difference in signed and absolute differences for RBC parameter was statistically significant (p=0.045). Similarly, 16 out-of-limit events were identified on analysis of Cusum chart of signed differences. Whereas only 12 out-of-limit events were identified on analysis of Cusum chart of absolute differences.

Conclusion: Present study show that a QC strategy of daily planned repeat sample testing can improve the out-of-control error detection ability simply by using signed difference of repeat sample result and applying usual Westgard rules.

Keywords: Levey–Jenning's Chart, Westgard Rules, Cusum Chart, Hemogram, Quality Control

Introduction

For a patient's health status, baseline CBC parameters and changes in these parameters is a most sensitive indicator. To cater increasing demand of more qualitative and quantitative CBC test reports, in past few years various technical advancement has taken place in hematology analyzers. Most of the hematology laboratory routinely uses automated hematology analyzer, for complete blood counts (CBC). A well calibrated hematology analyzer provides average characteristic features of the blood cells.[1] In order to provide reliable result for every sample, measures for internal and external quality control (QC) must be in place. Commercially available control material for internal quality control is expensive and may not always be available and feasible to use as they have a limited shelf life. Use of patient sample is more feasible and cost-effective alternative approach for various quality control measures. Blood specimens in anti-coagulant, ethylenediaminetetraacetate (EDTA), were found stable when refrigerated at 4 to 8°C for at least 24 hours.[2] The anti-coagulated retained blood samples were used in few previous studies for internal quality control.[3]

Quality control is used as a measure of precision to know how well the analytical system reproduces same results over time with the minor variation in operating conditions. In laboratories, internal QC are designed to detect error and deficiencies in analytical process to correct it prior to release of patient investigation results. Specimen samples for QC are sample that may be commercial or in-house prepared and resembling patient sample in their composition, matrix, viscosity turbidity and color. During the testing process QC samples are inserted in analytical runs and treated same as patient sample in same operating conditions.

QC samples are usually run after the service or calibration of instrument, change of reagent lot, when results seem inappropriate and beginning or change of shift. For an error free laboratory using automated hematology analyzer there is a need for appropriate QC measures to be in place.[4] The author Hu X et al has concluded that the hematology analyzer reports can be made reliable only by improving the laboratory quality management.[5]

Methods for quality control data interpretation involve both graphical and statistical methods. In the year 1950, Stanley Levey and, E. R. Jennings suggested, for quality control in clinical laboratory, Shewhart individual control chart can be used. They measured data point distance from mean in standard deviation and plotted a graph, now this graph is named as Levey–Jennings chart.[6] In year 1981 and 1986 J. O. Westgard, P.L. Barry, and M.R. Hunt suggested a Multi-rule Shewhart Chart. Westgard rules suggested, whether the results from the samples can be released, or need to be rerun on the basis of results of control material.[6,7] In Levey-Jennings control chart, along the X-axis dates of tests run are plotted and on the Y-axis control values are plotted. On the Y-axis, mean and standard deviation limits of 1s, 2s, and 3s are also marked. The presence of random error and trend or shift in calibration can be easily detected by inspecting the pattern of plotted data points. In a correctly and well operating system, repeat run of same control sample produces a Gaussian distribution. Approximately 66% of data points should fall between the +/- 1s ranges and distributed evenly on either side of mean. In a correctly operated system 95% data points lies between +/- 2s ranges and 99% between +/- 3s limits. Generally, the data point of +/- 2s limits are considered as a warning limits. A control material value between 2s and 3s is an indication for repeat analysis. The +/- 3s limits are rejection limits. When a data point falls outside of these limits, the test run should be stopped, patient results withheld, and the test system should be investigated.

In year 1954, the Cusum chart (cumulative some chart) was invented by British statistician ES. Page.[9] The Cusum chart is plotted by using cumulative sum of deviations, for individual measurement, from the target. The small shift in process mean can be monitored by Cusum chart.[9]

Some authors had reported that a repeat patient's fresh blood sample testing for daily QC test is a feasible and cost-effective method.[10] Our hematology laboratory participates once in every three months in the national EQAs program conducted by AIIMS, New Delhi and past performance was satisfactory. In our hematology laboratory random repeated sample testing method is used for daily QC.

The present study was designed to asses and re-establishes the utility and efficacy of in house random patient samples as a regular QC practice.

Materials and Methods

The present study was conducted at an Emergency Lab (Hematology Section) of a tertiary care hospital from 1 January 2020 to 3 September 2020. In the laboratory hemogram was done on five-part hematology analyzer (Model XT -2000i, Sysmex Corporation, Japan).

For quality control (QC) of hematology section a random repeated patient's sample technique was practiced, the difference between present and previous result of the sample was calculated. The differences for Hemoglobin, Red Blood Cells Total Leukocyte count and Platelet count were noted. The mean and standard deviation of difference were calculated, with and without considering the plus (+) and minus (-) sign. Levey–Jenning's chart (LJ chart) of the signed and unsigned differences were plotted for all these four hemogram parameters, Cusum chart was also plotted using the following formula.

For Cusum High:

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$$SH = SH + \left(\frac{(X-\mu)}{SD}\right) - KFracH$$

For Cusum Low:

$$SL = SL + \left(\frac{(X-\mu)}{SD}\right) + KFracL$$

In our case KFracH and KFracL were 0.5.

The performance of absolute difference was compared with the signed differences as a QC tool. All the calculations and graph were plotted using Python (version-3.8.13) and Jupyter Notebook (vesion-6.0.3). A z-test for difference in two proportions was used to assess the statistical significance of percentages of errors observed. A p value of less than 0.05 was considered as statistically significant.

Results and Observations:

There are in total of 246 differences included in the present study. A total of 49 violations of west guard rules observed if LJ Chart of signed differences was analyzed. Whereas a total of 30 violations of Westgard rules were observed on analysis of absolute differences. The details of Westgard rules violations is illustrated in Table 1, (the difference in signed and absolute differences for RBC parameter was statistically significant P=0.045)

Similarly, 16 out-of-limit events were identified on analysis of Cusum chart of signed differences. Whereas only 12 out-of-limit events were identified on analysis of Cusum chart of absolute differences Table 2. However, the difference in out of limit events of signed and absolute differences

Table 1.The Details of Westgard Rules Violations

	RBC		Hb		TLC		PLT		Total
	Signed	Absolute	Signed	Absolute	Signed	Absolute	Signed	Absolute	
1_{2S}	5	2	4	2	6	4	8	6	37
2_{2S}	2	0	0	0	0	0	0	0	2
1_{3S}	8	4	6	6	6	4	4	2	44
Total	15	6	10	8	12	8	12	8	79
N=246	6.09	2.44	4.06	3.25	4.88	3.25	4.88	3.25	32.11
P Value	0.045		0.632		0.360		0.360		37

Table 2.Out-of-Limit Events Identified on Analysis of Cusum Chart of Absolute and Signed Differences

	RBC		HB		TLC		PLT		Total
	Signed	Absolute	Signed	Absolute	Signed	Absolute	Signed	Absolute	
Out-of-limit events	0	2	4	4	2	4	10	2	28

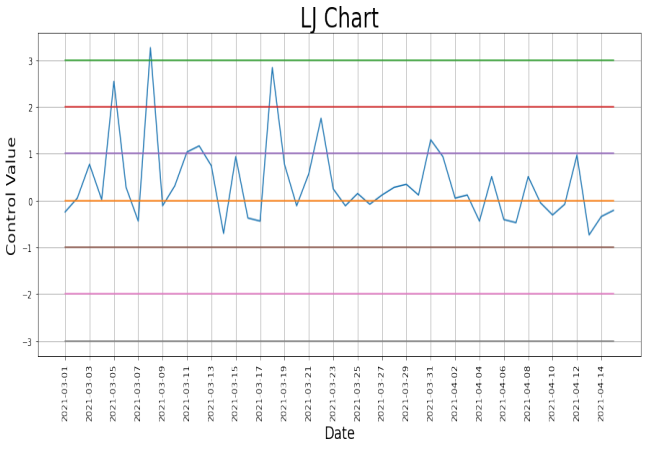

Figure 1(A).Lj Chart of Platelet Parameter Made Using Absolute Differences

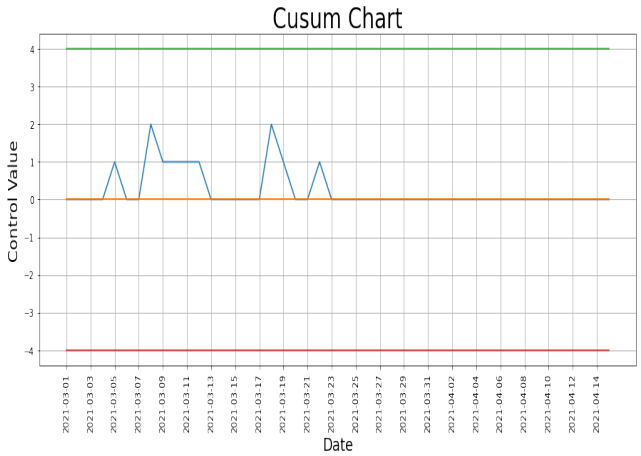

Figure 2(B).Cusum Chart of Platelet Parameter Made Using Asolute Differences

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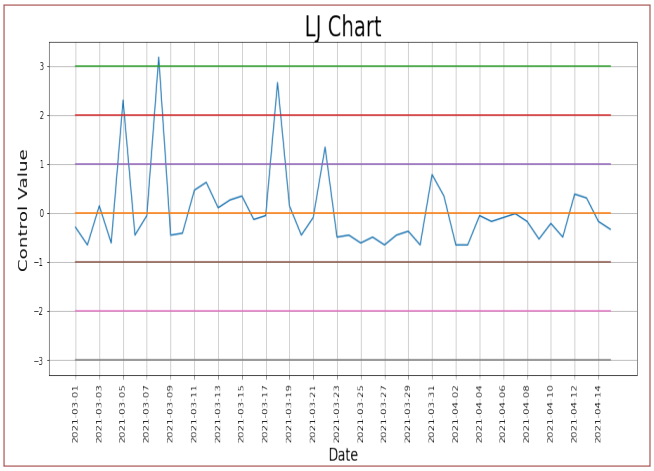

Figure 2(A).LJ Chart of Platelet Parameter Made Using Signed Differences

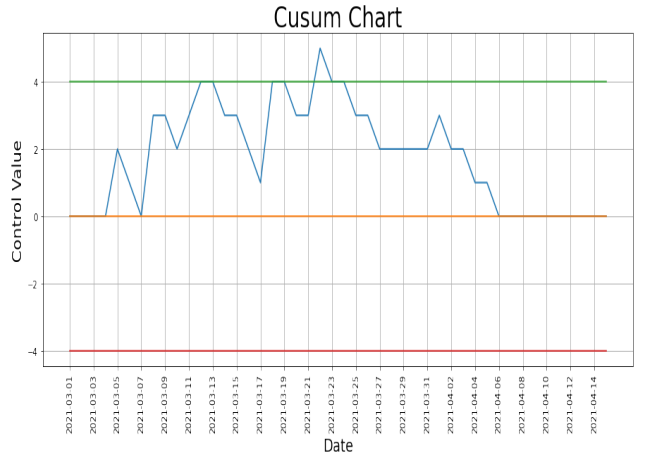

Figure 2(B).Cusum Chart of Platelet Parameter Made Using Signed Differences

was statistically insignificant. The representative Westgard rules violations on LJ chart and corresponding Cusum chart are illustrated in Figures 1 and 2.

Discussion

Although use of automated hematology analyzer speedup the process and eliminate human error, it is not free of error. Briggs et al.[11] and Curtis A et al.[12] stated that results of hematology analyzers has more variation in platelet count, than hemoglobin and WBC counts [11,12] Cembrowski and Parvin et al. had reported that incidence of error is maximum in RBC indices ((MCV, MCH and MCHC). The RBCs shows changes in a short period of time. The RBC indices are derived from hemoglobin, RBC count and hematocrit, and alteration in these factors results in imprecise results of the RBC indices.[11,13] Saxena R.[14] has reported that the fluctuations in the voltage, presence of dirt in tubing channel or sample aspirating, improper mixing of the blood sample, use of new improperly made or deteriorated reagents for CBC analysis are common causes of the random error in the hematology analyzer.[14] Many authors, Curtis A et al et al.[12], Briggs et al.[15] Hill VL et al.[16] Fernandez et al.[17] Singh T.[18] Have correlated results of hematology analyzer with peripheral blood smear and they observed that the misinterpretation of hematology analyzer results are due to morphological changes in blood cells.

Our study results show in a total of 246 repeat sample differences, a higher rate[49] of Westgard rules violations was observed in L.J. Chart of signed differences, whereas lower rate[30] violations of Westgard rules were observed on analysis of absolute differences Table 1. The difference in signed and absolute differences for RBC parameter was statistically significant (p=0.045). Similarly, 16 out-of-limit events were identified on analysis of Cusum chart of signed differences. Whereas only 12 out-of-limit events were identified on analysis of Cusum chart of absolute differences. The difference in out-of-limit events of signed and absolute differences was statistically insignificant Table 2. These result shows that, analysis of signed differences has more error detection rate compare to absolute differences, both in Cusum chart and in LJ chart. The LJ chart has higher error detection rate compare to Cusum chart.

In the year 1966, Frank Dutra introduces repeat whole blood sample of two successive days in place of control sample as Q.C. measure.[19] In subsequent years many authors[20,21,22,23] assessed the utility of repeat sample measurements as a tool of quality control. They independently computed and used their suitable control limits.

Cembrowski GS et al.[13] systematically studied the suitability of retained patient specimens as internal quality control material. They calculated the differences of replicated retained patient specimen. They used computer simulations technique to calculate probability of false rejection and probability of error detection. They concluded that if at-least two of the three differences for any CBC parameters exceed more than two standard deviations limits there will be a good probability of analytical error.

In the present study we have used the difference in two readings of randomly chosen patient's samples. We used mean and standard deviation of these differences to implement Westgard rules. We have used differences to make Cusum chart. There are previous studies comparing LJ chart and Cusum chart. The authors Yadav et al.[24] had plotted LJ chart and Cusum chart by using value of control sample and reported that Cusum chart is more sensitive in detecting systematic error as compared to Westgard rule on LJ chart, whereas Westgard rule have a greater chance to pick-up random error.[24] In the present study, LJ chart and Cusum chart were plotted by using signed and absolute difference of repeat sample.

There are other methods of internal quality control using information from patient's samples. In the year 1974,

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Brain Bull[25] proposed a reliable and cost-effective method of quality control called Bull's algorithm, by using patients test results simply. In the year 1974, Jerome Nosanchuk and Arthur Guttmann[26] suggested for detecting systematic error or analyzer error, individual patient result should be compared with their previous result known as delta check and when elapsed time is also taken in account it is called rate check.26

Our study result shows use of patient's fresh blood sample for repeat sample testing for daily QC is a good practice; it can be used on a regular basis as it is cost effective and feasible. It can pay a good role in error detection of hematology analyzer results, prior to reporting of patient's sample test results.

Conclusion

For internal daily quality control, LJ chart and application of Westgard rule on repeated sample testing of patients' fresh blood sample is feasible and cost-effective method. It can play a good role in error detection of hematology analyzer result, prior to reporting of patient sample test results. Present study show that a QC strategy of daily planned repeat sample testing can improve the out-of-control error detection ability simply by using signed difference of repeat sample result and applying Westgard rules.

Conflict of Interest: None

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