Original Article

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# Use of Patient Samples for Quality Control in Hemogram: An Experience from a Tertiary Care Centre in Southern India

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## ABSTRACT

**Background:** Quality control (QC) is an integral part of hematology practice. Various commercial controls are available and routinely used. A complementary method is to use patient sample in various ways especially in a resource limited setup.

**Methods:** This study was performed in a tertiary care hospital in southern India with 12 laboratories, four using hematology cell counters and eight other smaller side-labs performing Hemoglobin (Hb) estimation by Sahli's or Drabkin's method.

The QC tests performed using patient samples were: a. Daily - Average of numbers (AON) of RBC indices of 250-270 samples. These were compared with Levey-Jennings (LJ) charts of commercial controls b. Weekly - Replicate Test (RT) using a single sample for various levels of Hb, WBC and platelet counts; Duplicate Test (DT) using 10 samples; Correlation Check (CC) of 10 samples and Weekly AON c. Monthly - Deviation Index (DI) using one sample for inter-method/ instrument comparability among participating labs.

**Result:** In daily AON, the graphs of RBC indices did not show significant difference with LJ charts of commercial controls. The co-efficient of variation in RT for various levels of Hb, WBC and platelet counts were in the acceptable range. The precision was higher for Hb and least for low platelet counts. In DT, an occasional random error was detected. In CC, monocytosis flag did not correlate with peripheral smear findings 66.6% times. The DI using z-score showed satisfactory performance among various labs.

**Conclusion:** Use of patients' blood sample was helpful for internal QC and inter-method/ instrument comparability.

***Keywords:*** *Hemogram, Patient Sample, Quality Control, Levey – Jennings Chart*

## Introduction

Hematology analyzer based complete blood counts (CBC) are routinely ordered tests. A well calibrated hematology analyzer provides the *average* characteristic features of a cell.[1] In order to ensure that every sample gives the desired result; adequate quality control (QC) measures must be in place. It may not always be feasible to use commercially available controls as they are expensive and have a limited shelf life. An alternative approach is to complement it with use patient sample for various quality measures. When refrigerated (4 to $8^0$ C) the ethylenediaminetetraacetate (EDTA) anti-coagulated blood specimens were found stable. Many studies had used anti-coagulated retained blood samples for the purpose of internal quality control (IQC).[2] This study was undertaken with the objective of re-establishing the utility and efficacy of patient samples in regular QC practice and for performing inter-method and inter-instrument comparability among the various participating labs.

## Materials and Methods

This was a cross-sectional study conducted in a large tertiary care hospital in southern India that has several buildings and blocks. There are four laboratories [a main hematology lab, two Out-Patient Department (OPD) hematology labs and a medicine department lab] using hematology analyzers and 8 other small side-laboratories measuring hemoglobin (Hb) by manual method. These small labs are used for initial Hb estimation and samples with abnormal values are sent for repeat testing to one of the hematology labs (i.e., main lab and OPD labs). Ten 5-part hematology analyzers (XS1000i, XT1800i, XT-2000i, Sysmex Corporation, Japan) were included in the study for performing the daily, weekly and monthly quality control methods. Routine, fresh EDTA blood samples which were adequate in quantity and grossly appearing normal (non-clotted, non-hemolyzed, non- icteric, non- lipemic), for which CBC reports had been generated were chosen after noting their counts. For monthly inter-method/instrument comparability, the eight side labs performing manual Hemoglobin (Hb) estimation by Sahli's or Drabkin's method were also included. The QC tests performed were in accordance with the WHO lab manual of hematology (WHO/LAB/98.4).[3,4]

A. The following tests were done on a daily basis: (i) Average of numbers (AON) of RBC indices by using patient samples. The values of RBC indices were exported after interfacing of equipment with hospital information system (HIS). The target value

was established from the average of daily mean of initial two weeks i.e., 11 full working days. The standard deviation (SD) was calculated and target value +2SD and -2SD was taken as upper and lower limit respectively. The number of samples analyzed daily was 250 on an average. The values of daily means of MCH, MCHC and MCV plotted against the target values. (ii) Levey- Jennings (LJ) charts by using commercial controls were plotted daily for various CBC parameters by running tri-level controls depending on the availability. The target value, upper and lower limit were noted from the information provided by the manufacturer.

B. The following tests were done on a weekly basis: (i) Replicate Test (RT) was done by running a single random EDTA sample 10 times consecutively. Different random samples were chosen by convenient sampling for low, high and normal levels of Hb, white blood cells (WBC) and platelets (PLT) based on the reference range for a healthy adult as normal Hb: 120-170g/L, normal WBC: 4-11x$10^9$/L and normal platelet count: 150-400x$10^9$/L.[4] The mean, SD and co-efficient of variation (CV) for the values were calculated. (ii) Duplicate Test (DT) was done by taking 10 random samples which were run in two separate batches on the same day i.e., once in the forenoon and later in the afternoon. The mean and SD were calculated for the differences between the 2 runs. The *P* value was calculated using the 'paired t-test' to assess the significance of the difference between the 2 runs. DT indicated random errors if any. (iii) Correlation Check (CC) was done by choosing 10 random flagged samples and cross checking the flags with their corresponding peripheral smear (PS) report. For those that did not correlate with the flagging, the actual PS examination finding that was wrongly flagged was tabulated. (iv) Weekly AON was calculated from the daily AON to eliminate the effect of any day-to-day variation of patient profile. The AON over a period of time showed the presence or absence of systematic errors.

C. The test done on a monthly basis was Deviation Index (DI). This was done by running a single random EDTA sample in hematology analyzers as well as by the other manual methods being done in the 8 small side-labs. Sample volume of at least 2ml was taken and it was run by the lab personnel. The Z-score for Hb, WBC, RBC, MCHC and PLT for the analyzers and for Hb for the side-labs was calculated. Weighted SD was calculated by eliminating the value which was more than 2 SD and Z-score was re-calculated. The main hematology lab was the coordinating lab and other labs were participant labs for the monthly inter-method/ inter-laboratory harmonization. Regular feedback was given to the participant labs.

## Statistical Tests

Descriptive statistics were used. For AON, mean with SD were used. For RT, co-efficient of variation (CV) was expressed as median with range. For DT, SD was used and paired t-test was used for calculating the significance and *P* value < 0.05 was considered significant. For DI, Z-score was used which was given by the standard formula $z = \frac{x-\mu}{s}$ [z=z score, x=observed value, μ= mean and s= SD]. It was used to classify the performance of the participating laboratories and interpreted as follows: ± 0 – 2: Satisfactory, 2 - 3: Borderline and >3: Unsatisfactory. The participants were said to have persistent unsatisfactory performance if the value obtained by adding six of their recent Z scores and multiplying the sum with 6 was more than or equal to 100.

## Result

**Average of Numbers (AON):** The graphs plotted showed that the majority of RBC indices given by automated hematology analyzers were falling within 2SD of the target value except for the mean of MCV and some means of MCHC. The In daily AON charts were compared with the LJ charts of RBC indices of commercial controls. No significant differences were seen between the graphs of commercial controls and patient samples for the respective indices. An example of LJ charts showing daily AON and corresponding LJ chart using commercial control for a particular month is illustrated in Fig 1A and 1B respectively. These charts helped in identifying an occasional random error. No systematic errors were identified. No differences were seen between the daily and weekly AON using patient samples.

**Replicate Test:** CV for low, normal and high levels Hb, WBC and PLT on all the counters over 20 months is shown in Table1.The median CV for low levels of Hb, WBC and PLT was higher than the corresponding normal and high levels indicating that the machine had more precision for normal and high values than low values. The median CV for Hb was lower than WBC which was lower compared to that of PLTs indicating that Hb values are more precise and PLTs show more variation compared to Hb and WBCs.

**Duplicate Test:** An example of DT is illustrated in Table 2. The value of sample 4 was lying outside -2SD; indicating random error during its processing. DT showing values

lying within and outside 2SD for Hb, WBC and PLT was compiled and the *P* value was calculated using paired t-test. The outliers indicated random error during the run of a particular sample but only paired t-test showed whether the difference between the runs was significant or not. Among the 69 outliers of the total 200 DTs only 8 were significant.

**Correlation Check:** Among the flags studied, neutrophilia, microcytosis, anisocytosis, PLT abnormal distribution, hypochromia and leukocytopenia showed correlation with PS findings all the times. The observation made on the reported PS to explain for the flags/interpretive messages that did not correlate with the PS findings is shown in Table 3.

**Inter-method/ inter-instrument comparison-Deviation Index:** Over the entire study period, DI showed the overall performance of the various participating labs and allowed inter-instrument and inter-method comparability. There were borderline performers for Hb estimation by various side-labs. Only one of the side-labs showed unsatisfactory performance but none of them showed persistent borderline or unsatisfactory performance.

**Table 1: CV expressed as median with range for low (L), normal (N) and high (H) levels of Hb, WBC and PLT obtained by RT during the entire study period.**

| Parameter | CV | Median | Range |
|---|---|---|---|
| Hb | L | 1.06 | 0.16-2.08 |
| | N | 0.69 | 0.44-1.31 |
| | H | 0.58 | 0.32-0.91 |
| WBC | L | 2.38 | 0.88-3.73 |
| | N | 1.16 | 0.23-2.13 |
| | H | 1.23 | 0.46-1.9 |
| PLT | L | 6.66 | 2.15-14.15 |
| | N | 2.22 | 0.78-7.31 |
| | H | 1.61 | 0.83-5.1 |

**Table. 2: DT for a particular week showing random error in the run of sample 4.**

| WBC | | | | |
|---|---|---|---|---|
| n | Run I | Run II | d | $d^2$ |
| 1 | 9.57 | 9.67 | -0.10 | 0.01 |
| 2 | 6.13 | 6.28 | -0.15 | 0.02 |
| 3 | 7.17 | 7.07 | 0.10 | 0.01 |
| 4 | 7.35 | 7.56 | -0.21 | 0.04 |
| 5 | 6.52 | 6.42 | 0.10 | 0.01 |
| 6 | 4.06 | 4.07 | -0.01 | 0.00 |
| 7 | 7.76 | 7.66 | 0.10 | 0.01 |
| 8 | 9.24 | 9.18 | 0.06 | 0.00 |
| 9 | 6.57 | 6.62 | -0.05 | 0.00 |
| 10 | 5.08 | 5.20 | -0.12 | 0.01 |
| 10 | ∑ $d^2$ | | | 0.13 |
| | | ∑ $d^2$/2n | | 0.01 |
| | SQRT ∑ $d^2$/2n | | SD | 0.08 |
| | | | 2SD | 0.16 |

**Table 3: Observations made on the Peripheral Smear (PS) for the flags that did not correlate.**

| S.No. | Flag | No. of times(%) flag did not correlate with PS | PS findings |
|---|---|---|---|
| 1. | Atypical Lymphocytes | 7(46.7%) | 3 – reactive changes<br>2 – degenerated WBCs<br>2 – shift to left |
| 2. | NRBC | 5(35.7%) | 2 – shift to left<br>2 – giant platelets<br>1 – degenerated WBCs |
| 3. | Eosinophilia | 1(7.7%) | 1-toxic change with normal eosinophil count |

| S.No. | Flag | No. of times(%) flag did not correlate with PS | PS findings |
|---|---|---|---|
| 4. | Leukocytosis | 2(25%) | 2 – presence of nRBCs |
| 5. | Anemia | 4(25%) | 2 – normocytic normochromic RBCs with polychromatophils<br>2 – crenated RBCs |
| 6. | Thrombocytosis | 3(25%) | 2 – crenated RBCs<br>1 – fragmented RBCs |
| 7. | Immature Granulocytes | 5(36%) | 5 – toxic change without left shift |
| 8. | Thrombocytopenia | 5(38.5%) | 4 - adequate in clumps<br>1 – large platelets |
| 9. | Lymphocytosis | 1(14.3%) | 1 – normal distribution for the age (peadiatric sample) |
| 10. | Monocytosis | 2(66.7%) | 2– shift to left |

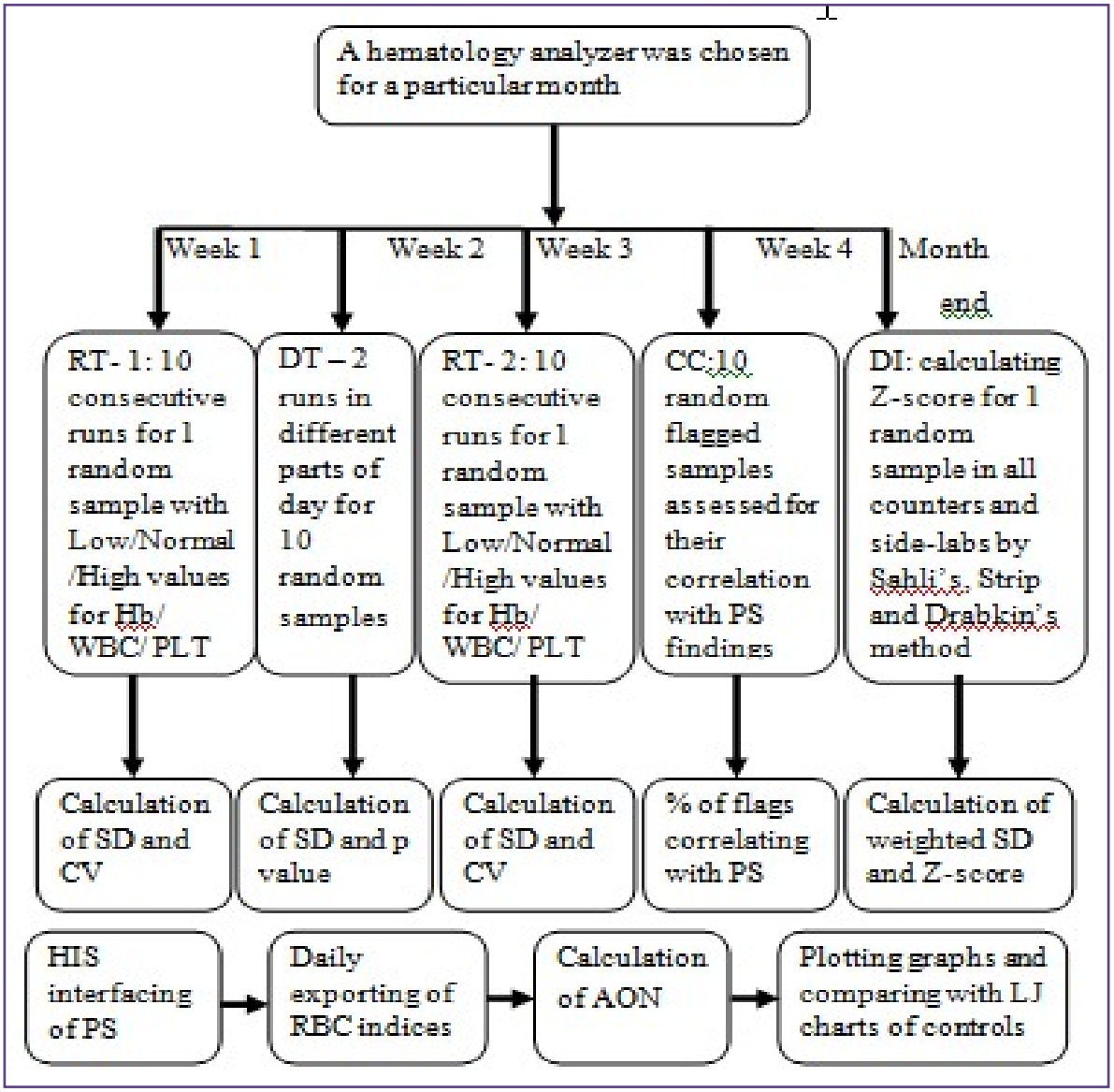


**Fig. 1 : The various QC tests performed in the study**

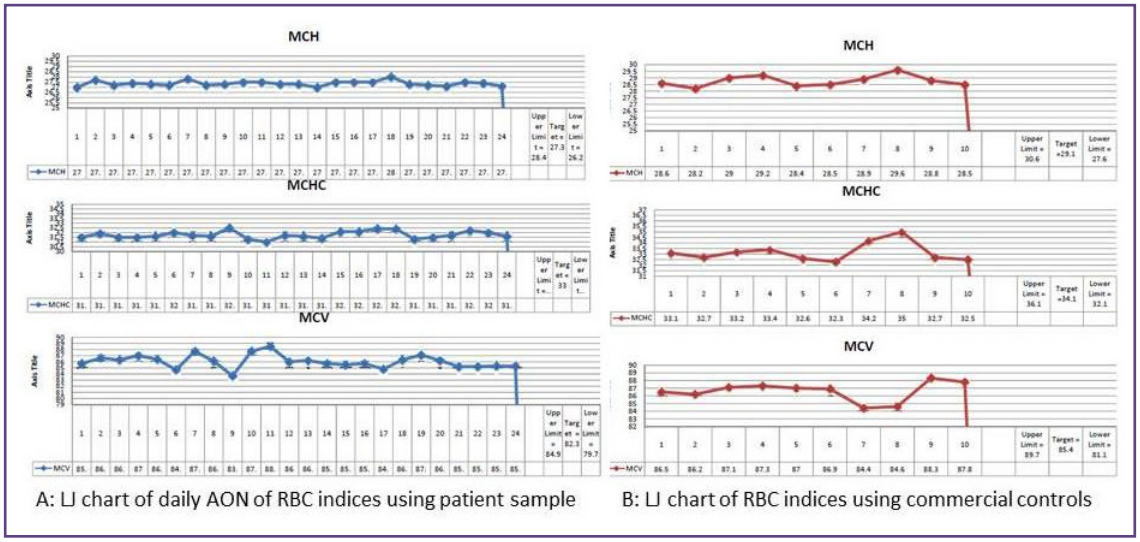


**Fig. 2: (A) LJ chart for RBC indices derived from AON of patient samples for a particular month compared with that (B) using normal level commercial control.**

## Discussion

The baseline CBC parameters and their changes act as one of the most sensitive indicators of an individual's health status. To cater to the increasing demands for more qualitative and quantitative CBC reports, several technical advances have taken place in the automatic hematology analyzers in the past few years. These various technical advances have also necessitated the need for appropriate QC measures to be in place for an error free laboratory practice.[5] The studies by Hu X et al established that the reports from a hematology analyzer can be made reliable only by improving the laboratory quality management.[6]

In a resource poor set-up, it is more feasible and cost-effective to use the fresh blood sample for QC measures rather than the commercial controls alone. In this study, daily and weekly AON, LJ charts by using commercial controls, RT, DT and CC were used for IQC. Although our main lab and two OPD hematology labs participate regularly in the national EQAS program, however to ensure inter-method and inter-instrument comparison among the labs across our institute, DI was used. During the study period, once in every three months all the three hematology labs participated in the EQAs program conducted by AIIMS, New Delhi. The 2ml EDTA blood sample received was processed in all the hematology analyzers of the participating labs. The slides of stained peripheral blood smears and reticulocyte preparation provided were examined and the findings were entered in the form sent along with the sample. The report form was duly sent back to the coordinating site for evaluation. During the entire study period the performance of hematology analyzers in the three participating labs was satisfactory.

The AON graphs plotted showed that the majority of RBC indices especially MCH given by automated hematology analyzers for OPD patient blood samples were falling within 2SD of the target values. MCV values showed fluctuations outside +2SD. For few months, the AON for MCHC was out of -2SD and it was correlating with the AON for MCV of those particular months which also showed AON falling outside 2SD. The fluctuations in MCV and sometimes in MCHC were explainable due to size changes of RBC on storage. Moreover, the target value and standard deviation has to be re-established periodically. Our findings are similar to observations by Cembrowski who stated that the amount of error is maximum in RBC indices (MCH, MCHC and MCV) as they are not directly measured but are derived by using number of RBCs, Hct and Hb. Hence the alteration in any of these factors resulted in imprecise results in the RBC indices. As Hct is the maximum affected parameter, it reflected in the imprecision in MCHC and MCV.[7] The LJ charts of tri-level commercial controls for the other parameters (Hb, WBC, and PLTs) were checked from lab records and were found to be within range re-affirming that there was no systematic error.

In replicate test, the median as well as the range of CV was higher for the low values of Hb, WBC and PLTs compared to the normal and high level values of the corresponding parameters. This implies that hematology analyzers are capable of more precise results for normal and high level values of Hb, WBC and PLTs than for the low levels. Likewise the median and range of CV was higher for PLTs compared to the same levels of Hb and WBC. This implies that the results for PLTs by hematology analyzers show more variation than those of Hb and WBC. The range of

CV for different levels of PLT and WBC counts were in acceptable range, as observed in a study by Briggs et.al.[8]

In duplicate test, occasional random errors were noted but all were not statistically significant. As RBCs have an inherent property to show changes over a short period of time, they were not included in this test. The most common causes for random error were either instrument related mainly due to fluctuations in the voltage, presence of dirt in the sample aspirating or tubing channel, technical errors like improper mixing of the sample and use of either deteriorated or improperly made new reagents for CBC analysis.[9]

In correlation check, atypical lymphocytes flagging which did not correlate with the PS had instead degenerated WBC, shift to left and reactive lymphocytes. As this flag includes a heterogeneous population of cells it does not show 100% correlation with PS. In the study done by Briggs et al, they assessed both atypical and abnormal lymphocytes flags together and this resulted in decreased false positive flags.[10] NRBC flagging that did not correlate in the PS showed instead shift to left, degenerated WBCs and giant platelets. In the study done by Briggs et al, they observed the flag to be more specific than sensitive.[10] The particular PS in which the 'Eosinophilia' flag did not correlate with the reported PS showed toxic change. As the neutrophils and eosinophils were analyzed in the same channel the coarse granularity in the toxic change was flagged as increased count despite normal eosinophil count. Those PS with which the 'Leukocytosis' flag did not correlate showed the presence of nRBCs. The nRBCs can be flagged as WBC as they are assessed by nuclear staining. Those PS with normocytic normochromic RBCs with crenated RBCs and polychromatophils were also flagged as anemia. In a study by Hill VL et al, the RBCs showed increased Interpretive Program (IP) flags by Sysmex XT – 2000i with increased age of the sample.[11] The PS in which the 'Thrombocytosis' flag did not correlate with the reported findings showed crenated and fragmented RBCs with normal range of PLTs. As in the literature, fragmented RBCs and crenated RBCs can be misinterpreted as PLTs due to their small size. All those PS that did not correlate with the 'Immature granulocyte' (IG) flag showed toxic change without left shift. In a study by Fernandez et al, there is a significant variation in the manual counting of immature granulocytes when compared to flagging by hematology analyzer. They gave a possible explanation of IGs when present in very small proportion are difficult to be evaluated by manual count of 100 WBCs.[12] 'Thrombocytopenia' flag that did not correlate, showed adequate number of platelets present in clumps majority of times and occasionally showed adequate large PLTs. As in the literature, large PLTs can be misinterpreted as RBCs by the counter and the platelet clumps are not assessed in the platelet channel and can be given as thrombocytopenia flag.[13] The PS with which the 'Lymphocytosis' flag did not correlate, showed an increase in lymphocytes, this was normal distribution of lymphocytes for that age, a three year old child. Those PS with which the 'Monocytosis' flag did not correlate had shift to left. In a study by Hill et al they observed that there is decrease in monocyte count in stored samples resulting in reduced number of monocytes over a period of time.[11]

Regarding the use of patients' fresh blood for inter-method/ inter-instrument and inter-laboratory comparability, occasionally the counters showed borderline performance for any one of the CBC parameters but there were no unsatisfactory performers or persistent borderline performers. Majority of times the hematology analyzers showed satisfactory performance for Hb estimation. The Hb estimation by rest of the methods i.e., Sahli's, Drabkin's and Strip method showed an occasional borderline performance. For the 18 DIs over the study period, one of the side-labs showed borderline performances for the highest number of times i.e., 6 times and another side-lab showed 4 borderline performances by the Drabkin's method and another side-lab showed unsatisfactory performance once during the study period.

In a study done by Min et al., they used Z-score for quantitatively analyzing the QC results by a new method called quantitative QC procedure (QQCP). In the QQCP method, they used Z-score to assess and represent the systematic errors quantitatively. They established the decision criteria as $\pm 3 \times \ddot{O}n$, where n is the number of assessed z-scores. This method helped the observers in quick analysis of the systematic error. They observed that it was possible in this method to detect the systematic error by less number of runs.[14] In a study by Park et al., they observed that it is more precise to establish an inter-instrument comparability by setting a cut off of 99$^{th}$ percentile than the 95$^{th}$percentile, which in turn reduced the number of unnecessary recalibrations of the instruments.[15]

## Conclusion

Use of patients' fresh blood sample was helpful for IQC, inter-method/inter- instrument and inter-laboratory comparability. The AON of RBC indices, RT and DT can be used for the IQC of hematology analyzers. The use of patients' fresh blood samples for these QC tests was feasible and cost effective and can be used on a regular basis complementing the use of commercial controls for optimal functioning of hematology laboratories.

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## Competing Interests
There are no competing interests by any other authors.

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