Original Article

Use of Retained Samples for Quality Control of Hematology Analyzers in Low-Resource Laboratories. A Comparative Study Between Retained Samples and Controls

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Abstract

Background: Quality control is essential for verifying the performance of hematology analyzers (HAs).While internal quality control (IQC) materials are recommended, however their use is often limited in low-resource settings due to high costs, short shelf life and logistical challenges. This study is done to evaluate the utility and effectiveness of using retained patient samples for daily quality control in hematology analyzers and to compare their performance with standard IQC data in terms of accuracy and precision.
Methods: Retained patient samples were tested in duplicate-once in the morning and again after overnight refrigerated storage (2–4°C). Results were compared with the corresponding IQC data in terms of calculating the coefficient of variation (CV%), standard deviation (SD) and performing statistical analysis for precision and reproducibility.
Result: Minimum and maximum range in our study for retained samples for Hb (gm/dl) was 4.60-17.40, WBC (10x3mm/dl) 3.20-23.70, RBC (10x6mm/dl) 1.54-7.72 , Platelets (10x6mm/dl) 77-656. Correlation coefficient between 2 values of retained samples : Hb (0.997), Platelets (0.994), RBC (0.967), WBC (0.993). SD calculated on daily value difference was: Hb (0.15), Platelets (9.61), RBC (0.17), WBC (0.30). There was significant correlation between the IQC and retained sample data on Paired t-test ($P < 0.05$).
Conclusion: P PAIR testing show significant correlation between retained samples and IQC and correlation coefficient is within limits. Retained human samples could be further studied and standardized for quality control use in resource-limited and remote settings where synthetic controls are cost-prohibitive.

Keywords: Coefficient of variation; Hematology analyzers; Internal quality control; Retained samples; Standard deviation

Introduction

Automation in laboratory medicine has increased the need for quality control and standardization of test parameters. Agencies such as NABL (National Accreditation Board For Testing And Calibration Laboratories) provide protocols to ensure consistent quality.

According to NABL guidelines, internal quality control (IQC) should be conducted twice daily, approximately 8 hours apart. Verification processes should be performed when a test is newly introduced and must include assessments for accuracy, precision, linearity, AMR (Analytical Measurement Range) and instrument checks.[1, 2, 3]

Hematology analyzers require periodic calibration and daily performance checks through QC (quality control) procedures. In low-resource settings, the use of commercial IQC materials is limited due to high cost and storage issues. EQAS (External quality assessment scheme) which is another cheap alternative for quality check is frequently received quarterly depending upon the program enrolled, therefore daily validation of quality is not possible.

As an alternative, retained patient samples offer a cost-effective and practical approach for quality monitoring. Thus, the present study was undertaken to determine the utility and efficacy of patient samples in regular QC of HA (hematological analyzer).

Materials and methods

This retrospective comparative analytical study was conducted at the NABL-accredited laboratory of tertiary care hospital, from January 2024 to December 2024 after taking approval from ethical committee of institute. A total of 365 whole blood samples collected in EDTA (Ethylene diamine tetra acetic acid) vials were tested in duplicate using the Beckman Coulter RBC DXH 800 analyzer and 3 level IQC samples were run 12-hourly for one year. Samples are selected randomly for duplicate runs as part of the quality control procedure.

Acceptance criteria for retained samples value difference was according to CLIA (Clinical Laboratory Improvement Amendments) guidelines, which states that the value of the first sample should either align with that of the second sample or fall within the specified acceptable range of variation.[4] Accepted range of variation for various parameters are as follow : Hemoglobin & RBCs: Must be within 4% of the target. WBCs : Must be within 10% of the target. Platelets: Must be within 25% of the target.

If a retained sample falls outside these percentages during a quality check, the lab's equipment may need to be repaired or recalibrated.

Retained patient samples were tested in duplicate—initially in the morning and again after overnight refrigerated storage at 2–4°C.[5, 6, 7] the samples received in evening usually between 4pm to 6pm are randomly selected and run on the HA in duplicate in the morning usually between 8am to 9am after routine maintenance but before running daily samples. The difference between values was recorded, and the coefficient of variation (CV%) and standard deviation (SD) of value differences were calculated on monthly basis.

Synthetic controls (IQC) were run on HAs 12-hourly before running patient samples according to NABL guidelines for 3 levels (L1, L2, L3). (IQC we were using were Coulter 6 cell control with short shelve life of 1 month) Monthly data for these controls were recorded in terms of CV% and SD.

First and second value of retained patient samples were compared with each other for precision and accuracy and CV and SD data of value differences of retained samples and CV and SD data of IQC were correlated with each other to know their correlation.

Statistical analysis

Data was entered into a computer software, and analytical statistical analysis was performed using computer software MedCalc.

Results

Duplicate Testing with Retained Sample: Data of 365 retained samples with minimum and maximum value with highest value of mean and SD. Table 1 compiles the data of retained samples with minimum value of parameter to maximum value included in study with their mean and standard deviation.

Table 1: Data of retained samples with minimum, maximum, mean, and standard deviation for various parameters.

Parameter	Minimum	Maximum	Mean	Standard Deviation
WBC	3.20	23.70	8.91	2.56
RBC	1.54	7.72	4.42	0.66
Hb	4.60	17.40	12.27	1.83
Platelets	77	656	237.82	83.81

Comparability Testing: It tests whether two values of a test produce consistent and interchangeable results. Figure 1 shows Scatter plots comparing initial and retested values for Hemoglobin (Hb), Red Blood Cells (RBC), White Blood Cells (WBC), and Platelet counts. Each plot includes a line of identity (dashed red) representing perfect agreement. High correlation between paired values supports the reproducibility and stability of retained samples used for internal quality control.

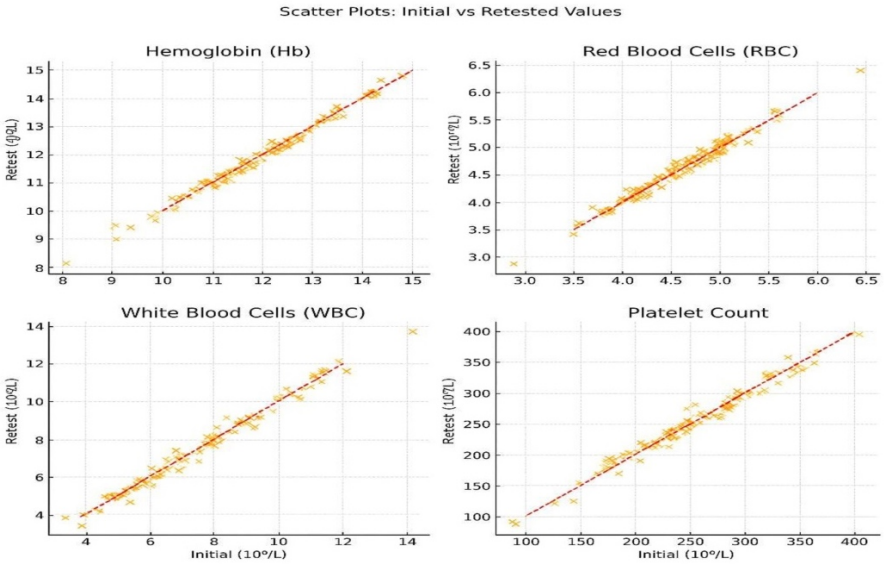

Figure 1: Scatter plots comparing initial and retested values for hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and platelet counts. Each plot includes a line of identity (dashed red) representing perfect agreement. High correlation between paired values supports the reproducibility and stability of retained samples used for internal quality control.

Bland and Altman analysis: Bland and Altman invented a graphical approach method to quantify agreement between two quantitative measurements by constructing limits of agreement. These statistical limits are calculated by using the mean and the standard deviation(s) of the differences between two measurements.[8] Our Bland-Altman analysis indicate that retest values of Hb, WBC, Platelets and RBC are within acceptable range. WBC shows slight downward bias and greater variability as compare to other parameters. In present study mean bias between 2 values of Hb, platelets, RBC and WBC is -0.04, 0.32, -0.03, -0.03 and limit of agreement for Bland–Altman analysis between initial and retested values of retained samples was -0.26 to 0.33, -19.53 to 18.90, -0.30 to 0.36 and -0.57 to 0.63.

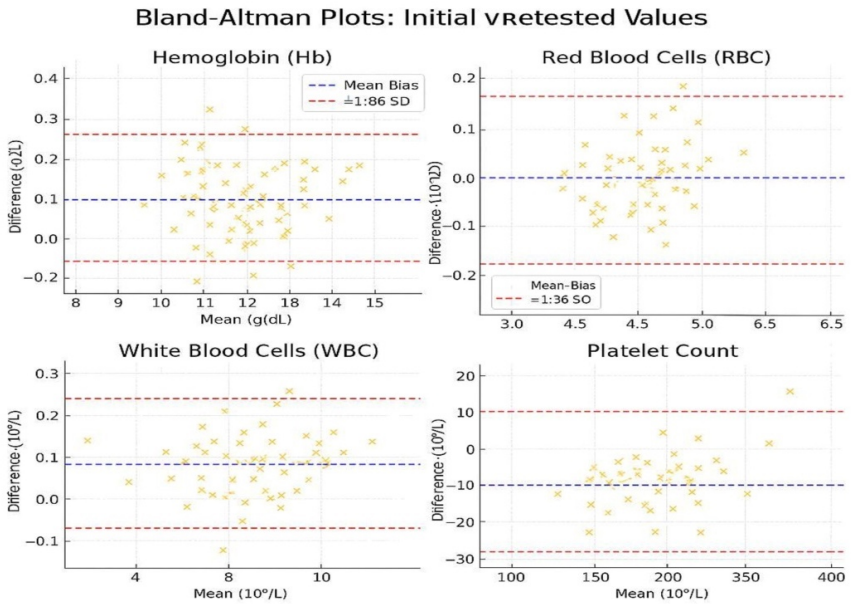

Figure 2: Bland–Altman plots showing agreement between initial and retested values. Central line indicates mean bias; dashed lines indicate ±1.96 SD. All values were within acceptable limits.

Comparability testing check the reproducibility of data while Bland and Altman analysis show limit of agreement between 2

values means how much two methods of measuring the same thing can differ and still be considered acceptable.

3.Summary Table: Table 2 summaries the statistical comparison between initial and retested values of retained samples which are showing statistical correlation between 2 values.

Table 2: Summary of statistical comparison between initial and retested values.

Parameter	Correlation (r)	Mean Difference	SD	95%Limits of Agreement
Hemoglobin (Hb)	0.997	−0.04	0.15	−0.29 to 0.33
Red Blood Cells	0.967	−0.03	0.17	−0.30 to 0.36
White Blood Cells	0.993	−0.03	0.30	−0.57 to 0.63
Platelet Count	0.994	0.32	9.61	−19.53 to 18.60

Above data of retained samples show significance correlation between 2 values (initial and retested) with very minor to no shift and drift of results. Analytical data of variation difference of retained samples (mean, SD, median, IQR) is then statistically correlated in Table 3 with analytical data of synthetic controls.

Table 3: Summarizes comparison data of monthly IQC data with value difference percentage data of retained samples.

	Retained sample				Synthetic Control				Z	p-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR		
WBC Variation%	−0.40	3.57	0.00	−2.39–1.53	1.53	0.64	1.31	1.08–1.83	−4.845	0.001
RBC Variation%	−0.68	3.53	−0.44	−1.26–0.38	0.95	0.35	0.87	0.77–1.02	−7.569	0.001
HB Variation%	−0.30	1.28	0.00	−0.84–0	0.72	0.34	0.62	0.54–0.75	−6.807	0.001
PLT Variation%	0.23	4.16	0.00	−2.43–2.93	2.71	1.02	2.55	2–3.31	−4.672	0.001

Paired t-test: p-value less than 0.05 indicates significant correlation between the two variables. p-value is a result of a hypothesis test that is less than a predetermined significance (0.05) indicate that the observed data are unlikely to have occurred by chance if the null hypothesis were true.

Discussion

In our study we used data of BACKMAN COULTER DXR 800 (24074) analyzer using 365 samples while in similar study conducted by Grillone et al. [9] in 2013 using BC6800VS ABX pentra DX120 using 20 samples while Xiang et al. [10] used Bc-5000 VS XB-2100 with sample size 310 samples and Kahar MA [11] (2023) used MEK9100VSXP-sysmex using 150 samples. Minimum and maximum range in our study for Hb is 4.60-17.40, WBC 3.20-23.70, RBC 1.54-7.72, Platelets 4.60-17.40. Which are comparable with study conducted by Xiang et al having range 6.4- 17.5, 1.37–58.9, 1.81–5.67, 23–691. Table 4 compares the statistical values of our study with other studies in literature.

Table 4: The statistical values for comparability (reproducibility) studies obtained in the present study in comparison with similar studies in literature.

S. no.	Study and year of publication	Grillone et al. 2013[9]	Xiang et al. 2015[10]	Kahar MA 2023[11]	Present study
1	Instrument name	BC6800VS ABX pentra DX120	BC-5000VS XB-2100	MEK9100VSXP-100	BACKMAN coulter DXR 800 24074
2	Sample size	20	310	150	365
3	Range				
	Hb(g/dL)	0.5–20	6.4–17.5	1.2–21.0	4.60-17.40
	Platelet count(10^9/L)	6.1–2000	23–691	14–1233	77-656
	RBC count(10^{12}/L)	0.17–7	1.81–5.67	0.40–6.80	1.54-7.72
	WBC count(10^9/L)	0.14–200	1.37–58.9	0.30–189	3.20-23.70
4	R (correlation coefficient)				
	Hb	0.998	0.9985	0.9811	0.997
	Platelet count	0.973	0.9856	0.9759	0.994
	RBC count	0.987	0.9963	0.9917	0.967
	WBC platelet count	0.995	0.9991	0.9968	0.993

Imprecision within value difference in retained sample calculated as CV% was compared with other similar studies published in literature and results are compiled in Table 5. Our values are comparable with other studies conducted by Briggs et al.[12], Grillone et al. and others.

Table 5: Imprecision as CV% within batch compared with similar published studies.

S. No.	Within batch	Briggs et al. 2012[12]	Grillone et al. 2013[9]	Xiang et al. 2015[10]	Kahar MA 2023[11]	present study 2025
1.	HB					
	Mean	-	14	119	12.3	12.27
	SD	-	0.11	0.98	0.21	1.83
	CV	-	0.81	0.89	1.73	
2.	Platelet					
	Mean	-	274	290	307.9	237.82
	SD	-	5.03	4.34	13.53	84.65
	CV	4.0	1.84	3.13	4.94	
3.	RBC					
	Mean	-	4.71	3.8	5.00	4.42
	SD	-	0.04	0.07	0.05	0.06
	CV	-	0.92	1.7	1.10	
4.	WBC					
	Mean	-	9.28	9.94	9.10	8.91
	SD	-	0.16	0.21	0.16	2.59
	CV	8.6	1.70	2.10	1.96	

Hb:Hemoglobin, RBC:Red blood cell, WBC:White blood cell, CV: Coefficient of variation, SD: Standard deviation

In present study when we compared the statistical data of variation of retained samples with monthly synthetic control data run on the same machine within same period of time and P value is calculated. P value was less than 0.05 (P 0.01). which support our hypothesis that there is significant correlation between the both values.

Commercially available Synthetic controls used in hematological lab as standard material used for quality control in many labs also guidelines are given by NABL to use synthetic controls to maintain the quality of machine but there are many pitfalls in the use of commercial synthetic controls.

Pitfalls of synthetic controls: 1. These samples are usually manipulated to lengthen the shelf life; therefore, they may behave differently than patient samples. 2. The manufacturer target's limits are often very broad, and subtle changes in analyzer behavior may thus be missed. 3. It is difficult to procure IQC material particularly for resource poor labs due to high cost and difficult maintenance and storage.

Despite above pitfalls there are many benefits of using synthetic controls in QC samples most important is to judge the instrument precision over time (i.e., drift) using a Levey-Jennings graph. However, it must be kept in mind that, at the end of the shelf life, the quality of the control samples may deteriorate.

Benefits of Retained Samples Over synthetic controls: As retained samples are actual samples of patient results will be comparable to original patient samples under various conditions. It is an economically better alternative as compared to synthetic controls. Storage and availability is easier as compared to synthetic controls.

Extreme ranges are (high and low ranges) are most important from a medical point of view. In the present study, the samples for correlation studies were randomly selected and may not match the above recommendations. Daily IQC calibration may have masked variations. Delta check current results compared with previous results were not evaluated as a method of QC using patient samples in present study. Some parameters may show variability issues with storage like platelets which tend to clump with storage giving falsely low values of platelets. So, proper trained staff is required to minimize such errors by maintaining proper SOP (STANDARD OPERATING PROCEDURE) to run the sample after bringing it to room temperature and mix gently.

Conclusion

Maintaining quality in testing ensures reliable reporting of results, enabling treating physicians to assess patients' conditions more precisely and thereby improving patient care, which is the ultimate objective of quality in laboratory medicine.

The high cost of internal quality control (IQC) materials, coupled with stringent storage and handling requirements, renders the routine use of synthetic controls impractical in small-scale laboratories with low daily testing volumes. In such settings, the use of retained samples with systematic data documentation can facilitate the assessment of test reproducibility, thereby ensuring the reliability and consistency of reported results. Keeping in mind the pitfalls of IQC material and advantages of retained samples further studies and research can be done on this topic to provide cheap, easy alternative method for small-scale/low budget laboratories and in resource-poor setting, to maintain the quality of testing.

According to NABL guidelines, IQC material is the best source to evaluate the performance of HA, In small laboratories, where few patient samples are reported and it is difficult to analyze stable control materials either due to cost or storage issue, repeat patient tests might provide a useful control for monitoring variability.[4] It is always better to do something than nothing and every sample matters.

Abbreviations: AMR: Analytical Measurement Range, CLIA: Clinical Laboratory Improvement Amendments, CV: Coefficient of Variation, EDTA: Ethylenediaminetetraacetic acid, EQAS: External Quality Assessment Scheme, HA: Hematology Analyzer, Hb: Hemoglobin, IQC: Internal Quality Control, NABL: National Accreditation Board for Testing and Calibration Laboratories, RBC: Red Blood Cells, SD: Standard Deviation, SOP: Standard Operating Procedure, WBC: White Blood Cells.

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Competing Interests: Nil

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