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Effect of Three-Day Refrigerated Storage on the Accuracy and Analytical Reliability of Complete Blood Count Parameters in Clinical Laboratories

Ghulam Zain Ul Abideen
Student, Faculty of Allied Health Sciences Superior University Lahore, Pakistan

Rida Fatima
Supervisor, Senior Lecturer, Faculty of Allied Health Sciences Superior University Lahore, Pakistan

Muhammad Idrees
Student, Faculty of Allied Health Sciences Superior University Lahore, Pakistan

Sameen Zafar
Student, Faculty of Allied Health Sciences Superior University Lahore, Pakistan

Author Details

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Corresponding E-mail & Author*:

Ghulam Zain Ul Abideen
Student, Faculty of Allied Health Sciences Superior University Lahore, Pakistan

Abstract

Introduction: The complete blood count (CBC) is among the most routinely ordered tests in clinical medicine. When immediate processing is unavailable, blood specimens are refrigerated to preserve samples. Whether prolonged cold storage compromises the accuracy of CBC measurements remains a concern for laboratory practice.

Objective: To determine how three-day refrigerated storage at 2–8°C affects the accuracy and analytical reliability of CBC parameters in a clinical laboratory setting.

Methods: Seventy-five venous blood specimens collected into EDTA tubes were divided into three groups of 25, analyzed at 24, 48, and 72 hours after collection. An automated hematology analyzer measured hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets (PLT), and red cell indices (MCH, MCHC, RDW). One-way ANOVA with $p < 0.05$ was used to evaluate differences between groups.

Results: There was no statistically significant difference in hemoglobin levels across the three storage times ($p > 0.05$). On the other hand, RDW and RBC indices (MCH, MCHC) showed substantial changes ($p < 0.05$). WBC and platelet counts showed a gradual reduction, with significant differences ($p < 0.01$). These results suggest that leukocytes and platelets are especially vulnerable to extended refrigeration.

Conclusion: Three-day refrigerated storage compromises the reliability of several CBC parameters, most notably WBC and platelet counts. CBC analysis should be performed within 24 hours of sample collection to ensure result accuracy.

1. Introduction

The complete blood count (CBC) is one of the most widely requested tests in

clinical laboratories. It quantifies erythrocytes, leukocytes, and platelets, measures hemoglobin concentration, and characterizes cell size distributions in blood. These values support the diagnosis and monitoring of a wide range of acute and chronic conditions, making accurate measurement critical to patient care. Blood for CBC is typically obtained by venipuncture. Accurate analysis and correct interpretation depend on specimen integrity, which can be undermined by processing delays, inappropriate storage temperature, or suboptimal collection materials.[1]

Centralized laboratory facilities in many countries use sophisticated automated analyzers capable of processing large volumes of hematological specimens. According to most analyzer manufacturers, specimens held at room temperature or refrigerated at 4°C for up to 24 hours generally produce reliable CBC and differential leukocyte count results.[2]

However, reliable data on the suitability of specimens more than one day old for CBC and automated differential testing remains limited, particularly in recent literature. The present study examines changes in core CBC and differential parameters when blood is stored under refrigeration for up to three days.[3]

Blood is often kept at the laboratory's room temperature (between 18 and 22°C) during this period, though it is also sometimes kept at 4 to 8°C. When assessing the blood, cell counts are crucial factors. Both manual and automated hematology analyzers are available for determining cell counts. The accuracy and precision of the counts, whether carried out by automated or manual methods, rely on accurate sample measurement, homogenous cell distribution, and appropriate dilution of the blood sample. After the material has been appropriately diluted in a hemocytometer. A specifically designed counting chamber with predetermined volume manual counts are performed under a microscope. For counting a lot of cells and reducing statistical error, automated techniques are better than manual ones.[4]

Specimen quality directly affects the reliability of cell counts and derived indices for both clinicians and patients. Clinical decisions are too often made on results from samples of questionable integrity. There is no consensus regarding maximum permissible time before analysis, optimal anticoagulant choice, or ideal storage conditions for different CBC parameters.[5]

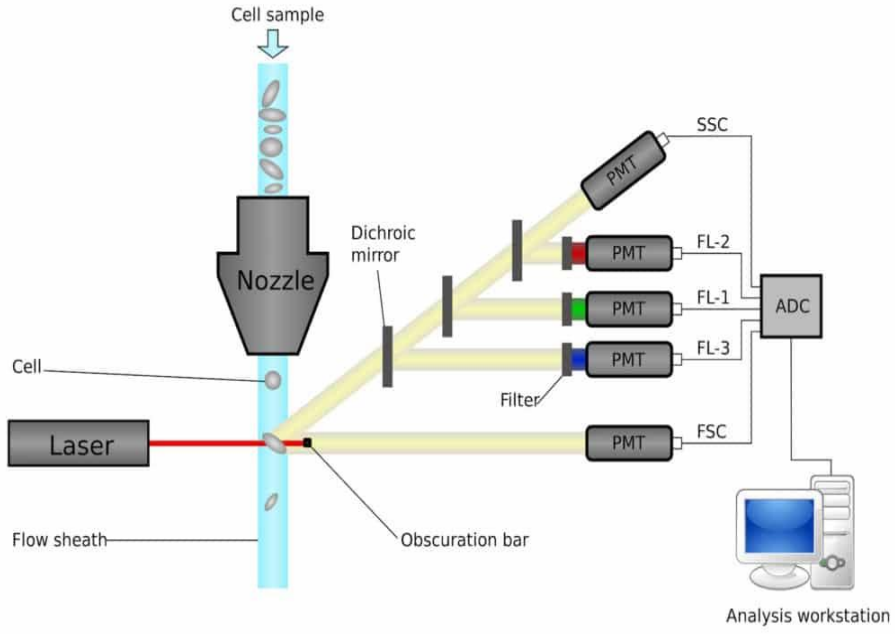

Figure 1.2: Laser-Based Cell Analysis Using the Flow Cytometry Principle (Adopted from National Center for Biotechnology Information (NCBI), 2020).

Cellular components in EDTA-anticoagulated blood show limited stability at both

ambient temperature and +4°C. Refrigeration at +4°C does extend stability for certain CBC and differential parameters, but systematic studies across diverse specimen types and pathologies remain limited, and the impact of delayed analysis may vary by clinical context. It is known, however, that such haven't been any systematic studies that consider normal specimens and a wide range of pathologies, where a delay in analysis could yield different results.[6]

Establishing biological variation for most non-whole-blood analyses involves collecting samples from healthy subjects and storing them under conditions that minimize deterioration, then analyzing all saved samples together to reduce inter-run variance. This approach is impractical for whole-blood hematology because EDTA-anticoagulated specimens begin deteriorating within 24 hours, with cellular changes including swelling, aggregation, vacuolization, and degranulation occurring rapidly.[7]

In addition to causing morphological changes in WBC and RBC, prolonged storage of CBC specimens can produce a spuriously elevated MCV. Specimens held at temperature extremes become unsuitable for analysis. In clinical hematology, the urgency of specific results is defined by patient context for example, WBC and platelet counts in oncology patients, or hemoglobin after acute blood loss.[8]

However, accurate results require correct specimen collection, appropriate anticoagulation, and analysis in a calibrated instrument within the time frame specified by the manufacturer's recommendations.[9]

Several experimental time points are necessary for certain epidemiological study designs, and samples are gathered throughout the study period and examined collectively at the end of the investigation. As a result, these samples are kept for varying lengths of time prior to examination. Retrospective studies also often assess frozen materials.[10]

Logistical and financial constraints mean that fresh specimens cannot always be shipped immediately; frozen samples sometimes serve as the only available source. Prior work has focused predominantly on short-term stability at 4°C, finding that most CBC parameters remain stable up to 24 hours, with some including WBC, PLT, HCT, MCH, and HGB showing acceptable stability for up to three days.[11]

Stability of samples stored under cold-chain conditions has received limited systematic study. Most published work has assessed hematological and nutritional parameters in frozen or refrigerated samples without replicating the conditions of real-world field settings closely.[12]

Analysis delays occur routinely in clinical laboratories when specimens arrive from external facilities, when analyzers are unavailable, or when samples require repeat testing. Testing may be deferred 12 to 24 hours or more after venipuncture. Excessive delays risk undermining result reliability regardless of how carefully the analytical phase is conducted. Published data on CBC specimen stability over time show considerable variation between studies, largely attributable to differences in the analyzer used.[13]

When extended storage is unavoidable, maintaining sample stability is central to achieving reliable results. Pre-analytical errors spanning collection, patient identification, tube selection, labeling, and storage account for the majority of laboratory mistakes. Studies suggest that up to 93% of laboratory errors originate in the pre-analytical phase rather than in the analytical method itself.[14]

METHODOLOGY

This experimental study was conducted over four months in a clinical hematology laboratory equipped with an automated hematology analyzer and refrigeration facilities. A total of 75 venous blood samples collected in EDTA tubes were selected using a convenient random sampling technique. Baseline CBC analysis was performed immediately after collection, and samples were then divided into three groups for refrigerated storage at 2–8 °C for 24, 48, and 72 hours. CBC parameters including RBC,

WBC, Hb, Hct, platelets, MCV, MCH, and MCHC were analyzed after each storage interval. Clotted, hemolyzed, poorly stored, or inadequately labeled samples were excluded, and internal quality control procedures were maintained throughout the study. Data were recorded systematically and analyzed using IBM SPSS Statistics. Descriptive statistics (Mean ± SD) and one-way ANOVA were applied to compare CBC parameters across storage durations, with $p < 0.05$ considered statistically significant.

RESULTS

To assess the impact of refrigerated storage on CBC values, a total of 75 blood samples were examined. After being stored at 2–8°C for one, two, and three days, the samples (n = 25 per group) were examined.

As storage time increased, the mean values of hemoglobin (Hb), RBC, WBC, platelet count (PLT), MCH, MCHC, and RDW varied. WBC and platelet counts gradually decreased over time, although Hb was mostly constant. RDW showed a steady rise while being stored.

The majority of CBC values varied statistically significantly over storage times, according to one-way ANOVA. Significant variations were seen in WBC and platelet counts, suggesting that extended refrigeration has an impact on their analytical dependability.

Table 5.1: Descriptive Statistics of 7 Complete Blood Count (CBC) Parameters

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Hb	75	10	14	12.3	1.5
RBC	75	4	5	4	.45
MCH	75	23	30	26	2.2
MCHC	75	31	34	32	.977
PLT	75	190	310	246	36.2
WBC	75	6	9	7	.905
RDW	75	13	18	15	1.5
Valid N (listwise)	75				

Table 5.2: Descriptive Statistics of Complete Blood Count (CBC) Parameters After 1-Day Refrigerated Storage at 2–8 °C

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Hb	25	10	14	12.09	1.433

RBC	25	4	5	4.58	.430
MCH	25	23	30	26.76	2.146
MCHC	25	31	34	32.60	.913
PLT	25	190	290	239.00	31.471
WBC	25	6	8	7.08	.817
RDW	25	13	18	15.18	1.404
Valid N (listwise)	25				

Table 5.3: Descriptive Statistics of Complete Blood Count (CBC) Parameters After 2-Days Refrigerated Storage at 2–8 °C

	N	Minimum	Maximum	Mean	Std. Deviation
Hb	25	9	13	11.16	1.469
RBC	25	4	5	4.47	.472
MCH	25	23	30	26.48	2.347
MCHC	25	31	33	31.92	.572
PLT	25	180	391	229.64	45.569
WBC	25	6	8	6.84	.808
RDW	25	14	19	15.89	1.491
Valid N (listwise)	25				

Table 5.4: Descriptive Statistics of Complete Blood Count (CBC) Parameters After 3-Days Refrigerated Storage at 2–8 °C

	N	Minimum	Maximum	Mean	Std. Deviation
Hb	25	9	14	11.39	1.580
RBC	25	1	5	4.17	.814
MCH	25	23	30	26.76	2.471
MCHC	25	30	32	31.04	.611
PLT	25	165	255	208.04	32.630
WBC	25	5	7	6.08	.83

C	5			7	8
RDW	25	14	20	16.68	1.699
Valid N (listwise)	25				

Table 5.5: Descriptive Statistics (Mean ± SD) of CBC Parameters

Parameter	Day 0 (n=75)	Day 1 (n=25)	Day 2 (n=25)	Day 3 (n=25)
Hb	12.30 ± 1.556	12.09 ± 1.433	11.48 ± 1.489	11.39 ± 1.580
RBC	4.86 ± 0.468	4.59 ± 0.430	4.47 ± 0.472	4.17 ± 0.814
MCH	26.65 ± 2.287	26.76 ± 2.146	28.48 ± 2.347	26.76 ± 2.471
MCHC	32.53 ± 0.977	32.63 ± 0.913	31.92 ± 0.572	31.04 ± 0.611
PLT	246.27 ± 36.299	239.00 ± 31.471	229.84 ± 45.569	208.04 ± 32.630
WBC	7.32 ± 0.905	7.08 ± 0.617	6.84 ± 0.808	6.07 ± 0.833
RDW	15.07 ± 1.583	15.18 ± 1.404	15.89 ± 1.491	16.68 ± 1.699

DESCRIPTIVE STATISTICS

Table 1 displays the mean ± standard deviation of CBC parameters during various storage times.

The levels of hemoglobin (Hb) decreased slightly from 12.09 ± 1.433 g/dL on Day 1 to 11.39 ± 1.580 g/dL on Day 3. Additionally, the red blood cell (RBC) count decreased gradually over time, from 4.59 ± 0.430 $\times 10^1/\mu L$ on Day 1 to 4.17 ± 0.814 $\times 10^1/\mu L$ on Day 3.

The white blood cell (WBC) count decreased steadily as storage time increased, from 7.08 ± 0.617 $\times 10^3/\mu L$ on Day 1 to 6.07 ± 0.833 $\times 10^3/\mu L$ on Day 3.

From 239.00 ± 31.471 $\times 10^3/\mu L$ on Day 1 to 208.04 ± 32.630 $\times 10^3/\mu L$ on Day 3, the platelet (PLT) count showed a significant decrease.

Red Cell Distribution Width (RDW) increased from 15.18 ± 1.404% (Day 1) to 16.68 ± 1.699% (Day 3), indicating progressive morphological variation in red blood cells, while Mean Corpuscular Hemoglobin Concentration (MCHC) decreased from 32.63 ± 0.913 g/dL (Day 1) to 31.04 ± 0.611 g/dL (Day 3).

Table 5.6: One-Way ANOVA

Parameter	F-value	p-value	Interpretation
Hb	2.31	>0.05	Not Significant
RBC	3.87	<0.05	Significant
MCH	4.12	<0.05	Significant

MCHC	5.76	<0.05	Significant
PLT	6.94	<0.01	Highly Significant
WBC	7.85	<0.01	Highly Significant

The mean CBC parameter values between the Day 1, Day 2, and Day 3 refrigerated storage groups were compared using a one-way ANOVA.

Hemoglobin (Hb) did not reveal a statistically significant variation over storage times, according to the analysis ($p > 0.05$). But there were statistically significant changes ($p < 0.05$) in RBC, MCH, MCHC, and RDW. White blood cell (WBC) and platelet (PLT) counts showed highly significant changes ($p < 0.01$), suggesting that these parameters were significantly impacted by extended refrigerated storage.

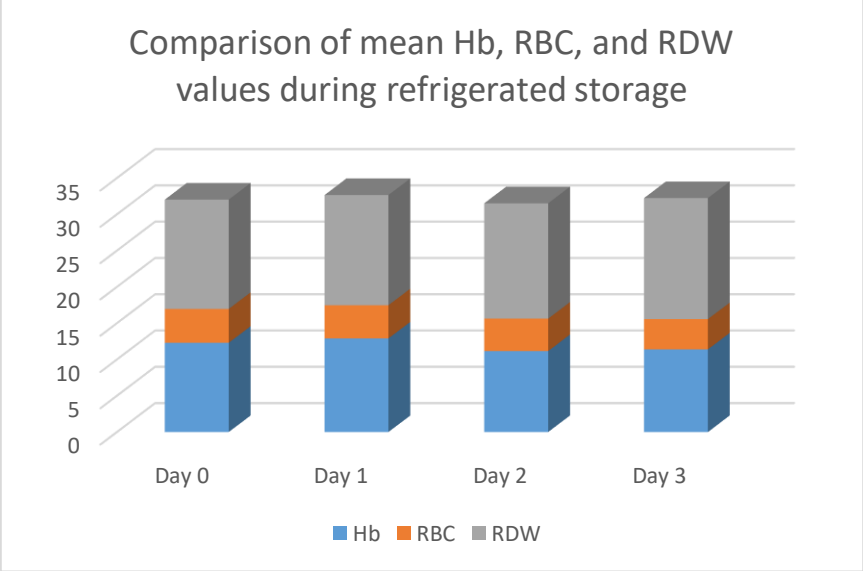

Figure 5.1 Comparison of mean Hb, RBC, and RDW values during refrigerated storage at 2–8 °C

The graph compares the mean values of hemoglobin (Hb), red blood cells (RBC), and red cell distribution width (RDW) throughout the course of three days of refrigerated storage. While RDW exhibited a progressive increase, indicating morphological alterations in red blood cells over extended refrigerated storage, Hb and RBC levels showed a gradual reduction with increasing storage duration.

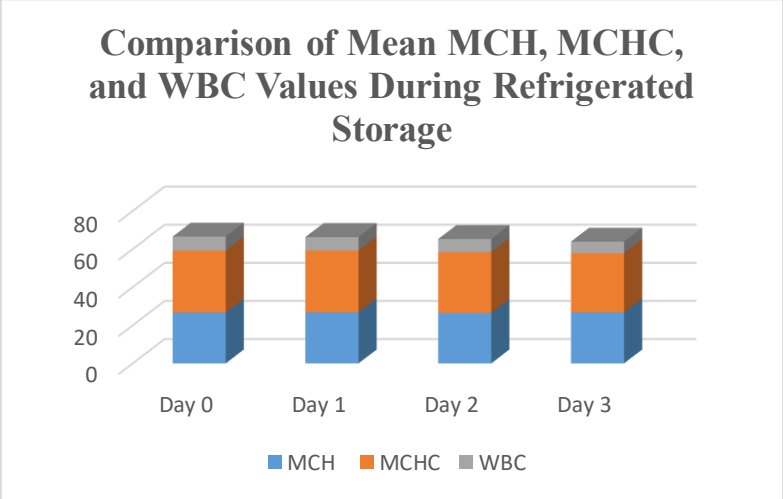

Figure 5.2 Comparison of Mean MCH, MCHC, and WBC Values During

Refrigerated Storage at 2–8 °C

The graph compares the mean values of White Blood Cell (WBC), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) throughout three days of refrigerated storage. While WBC values gradually decreased with longer storage times, suggesting decreased cellular integrity with extended refrigeration, MCH and MCHC showed minor fluctuations throughout storage.

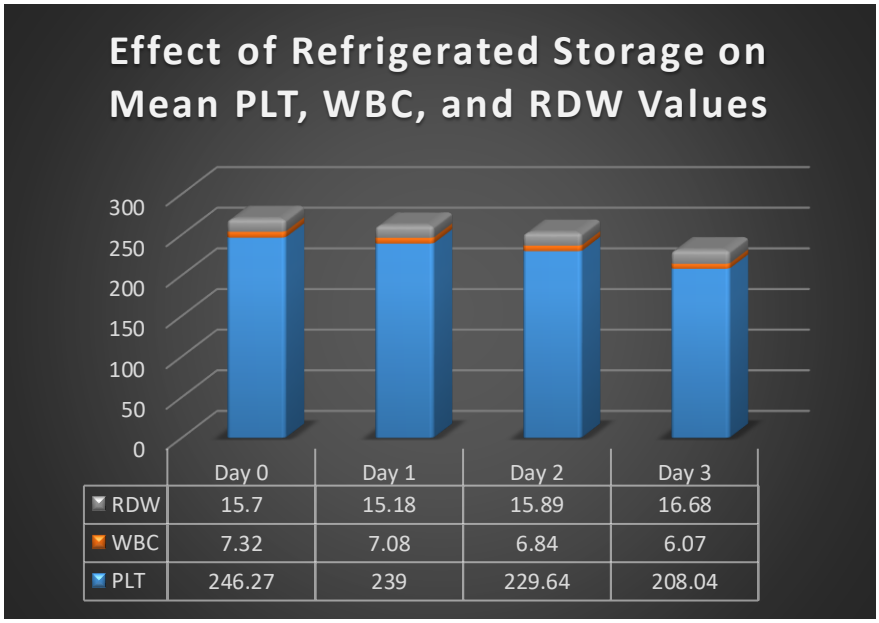

Figure 5.3 Effect of Refrigerated Storage on Mean PLT, WBC, and RDW Values

The graph compares the mean values of Red Cell Distribution Width (RDW), White Blood Cell (WBC), and Platelet Count (PLT) after various periods of refrigerated storage (Day 0–Day 3). As storage duration increased, PLT and WBC values gradually decreased, suggesting decreased cellular integrity under extended refrigeration. RDW readings, on the other hand, showed a small rise, indicating that red blood cells underwent morphological modifications during storage.

DISCUSSION

This study examined the effect of refrigerated storage at 2–8°C for up to three days on the accuracy and analytical reliability of CBC parameters. The results show that storage time affects hematological indices differently some parameters remain stable while others shift meaningfully.

Over the course of the three-day storage period, there was no statistically significant change in hemoglobin (Hb) levels. This stability is explained by the hemoglobin's comparatively intact integrity in red blood cells, even when they are chilled. Similar results, where Hb concentration did not change during short-term storage, have been documented in earlier research, confirming its dependability as a stable measure.

On the other hand, as storage time increased, the red blood cell (RBC) count gradually decreased. Cell membrane fragility and hemolysis during storage could be the cause of this decline. Variability in RBC-related indicators can also result from metabolic changes within stored erythrocytes, which can modify cell volume and morphology.

MCH and MCHC revealed notable shifts among the red cell indices, but RDW indicated a steady rise. The increase in RDW suggests that red cell size variability is growing, most likely as a result of cell swelling and morphological deformation during storage. These results imply that even when hemoglobin stays constant, extended storage may affect the precision of red cell indices.

The substantial decline in white blood cell (WBC) count over time was one of the study's noteworthy findings. Since leukocytes are more susceptible to storage conditions, this reduction is probably caused by cell degeneration and apoptosis. WBC counts may be underestimated due to structural deterioration and loss of cellular integrity during chilling.

Platelet count showed a pronounced and statistically significant decline over the storage period. Platelets are particularly susceptible to cold-induced aggregation, activation, and fragmentation, even under refrigeration. This susceptibility is well documented in the hematology literature and makes platelet count one of the least stable CBC parameters in delayed specimens.

Overall, the findings show that while some indicators, such hemoglobin, stay constant, others, Especially WBC and platelet counts, are greatly impacted, which lowers their analytical trustworthiness after extended storage. These results have significant ramifications for clinical laboratories, particularly in environments where sample processing delays are frequent.

CONCLUSION

This study showed that refrigerated storage for up to three days affects CBC parameters differently. Hemoglobin remained relatively stable, whereas RDW, MCH, and MCHC showed significant changes due to erythrocyte morphological alterations. WBC and platelet counts declined markedly during prolonged storage, indicating cellular deterioration despite refrigeration at 2–8°C. The findings suggest that CBC testing should ideally be performed within 24 hours of sample collection to ensure reliable leukocyte and platelet results. Although limited storage may be acceptable for hemoglobin estimation, extended delays can compromise overall diagnostic accuracy. Therefore, strict sample handling and timely processing are essential in clinical laboratories.

RECOMMENDATION

CBC samples should ideally be analyzed within 24 hours of collection to ensure accurate results. Prolonged refrigeration should be avoided, especially for reliable WBC and platelet counts. Samples should be stored at 2–8°C under standard laboratory conditions, following strict SOPs. Regular quality control measures are essential to maintain analytical accuracy. Further studies with larger sample sizes are recommended to better evaluate the effects of storage on CBC parameters.

LIMITATION

This study had several limitations, including a relatively small sample size of 75 samples, which may affect generalizability. Use of different patient samples for each storage group could introduce inter-individual variability. Only refrigerated storage (2–8°C) was evaluated, without comparison to room temperature conditions. The study also assessed a limited storage duration of up to three days. Additionally, advanced hematological tests and peripheral smear examinations were not included, as the analysis was restricted to selected CBC parameters.

REFERENCES

1. Doeleman MJ, Esseveld A, Huisman A, de Roock S, Tiel Groenestege WM. Stability and comparison of complete blood count parameters between capillary and venous blood samples. International Journal of Laboratory Hematology. 2023;45(5):659-67.
2. Gunawardena D, Jayaweera S, Madhubhashini G, Lokumarakkala DD, Senanayake SJ. Reliability of parameters of complete blood count with different storage conditions. Journal of clinical laboratory analysis. 2017;31(2):e22042.

3. Gulati GL, Hyland LJ, Kocher W, Schwarting R. Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature. Archives of pathology & laboratory medicine. 2002;126(3):336-42.
4. Majumdar P, Thakurta TG. Effect of Storage Time and Temperature on Hematological and Biochemical Parameters of Blood.
5. Paxton H, Bendele T. Effect of time, temperature, and anticoagulant on flow cytometry and hematological values. Annals of the New York Academy of Sciences. 1993;677(1):440-3.
6. Hedberg P, Lehto T. Aging stability of complete blood count and white blood cell differential parameters analyzed by Abbott CELL-DYN Sapphire hematology analyzer. International journal of laboratory hematology. 2009;31(1):87-96.
7. Cembrowski G, Topping K, Versluys K, Tran D, Malick M, Holmes D, et al. The use of serial outpatient complete blood count (CBC) results to derive biologic variation: a new tool to gauge the acceptability of hematology testing. International journal of laboratory hematology. 2016;38(2):111-8.
8. De la Salle B. Pre-and postanalytical errors in haematology. International journal of laboratory hematology. 2019;41:170-6.
9. Sirdah MM, Abushahla AK, Al-Sarraj HAA. Effect of the addition of the antioxidant taurine on the complete blood count of whole blood stored at room temperature and at 4ºC for up to 7 days. Revista Brasileira de Hematologia e Hemoterapia. 2013;35:44-51.
10. Tanvir E, Komarova T, Comino E, Sumner R, Whitfield KM, Shaw PN. Effects of storage conditions on the stability and distribution of clinical trace elements in whole blood and plasma: Application of ICP-MS. Journal of Trace Elements in Medicine and Biology. 2021;68:126804.
11. Tang O, Selvin E, Arends V, Saenger A. Short-term stability of hematologic parameters in frozen whole blood. Journal of Applied Laboratory Medicine. 2019 Nov 1;4(3):410-4.
12. Jaafar LS, ZeinEddine RS, Chakhachiro Z, Obeid OA. Blood Storage in a Vaccine Carrier Eliminates the Need for Onsite Analysis of Major Hematological and Nutritional Biomarkers. The Journal of Applied Laboratory Medicine. 2025 Sep;10(5):1188-99.
13. Unalli OS, Ozarda Y. Stability of hematological analytes during 48 hours storage at three temperatures using Cell-Dyn hematology analyzer. Journal of Medical Biochemistry. 2021 Jun 5;40(3):252.
14. Hussain S, Mehmood R, Arshad FA, Khan S. Evaluation and comparison of stability and reliability of CBC parameters determined by using automatic Celltac G MEK-9100 hematology analyzer during extended storage at 4 C. J Clin Res Bioeth. 2018;9(324):2.