CLINICAL LABORATORY

Impact of pre-analytical variables - temperature, agitation, storage duration, and blood-to-anticoagulant ratio - on complete blood count test reliability

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Abstract

Background. The complete blood count is a widely used medical laboratory test that is essential for both diagnosis and treatment decisions. While pre-analytic factors, such as storage temperature, transport conditions, blood volume anticoagulant ratio, and testing delay, might impact the accuracy of the results, the degree of this influence requires further quantification. In the present study, we aim to look more closely at these factors and how they affect complete blood count test readings.

Methods. This study evaluates the effects of temperature, agitation, storage duration, and blood volume anticoagulant ratio on complete blood count reliability using a repeated-measures factorial design. A total of 224 CBC runs were performed on the samples of eight participants. Samples were tested at baseline T0 and again at 4, 12, and 24 hours after storage at either room temperature (22°C) or refrigeration (4°C), with and without agitation, and at both optimal (2 mL) and suboptimal (1 mL) blood volumes.

Results. The duration of storage was the main factor determining CBC accuracy. After 12 h, WBC counts decreased significantly ($p < 0.001$, $\eta^2 = 0.129$). Platelet counts also declined ($p < 0.001$, $\eta^2 = 0.73$), with greater loss at 4°C (–8.1%) than at 22°C (–5.2%) by 24 h. MCV remained stable at 4°C but increased at 22°C ($p < 0.001$). Hemoglobin decreased at 22°C (−2.3%, $p < 0.05$), and RBC counts dropped modestly (−2.2%, $p < 0.05$). MPV showed only a minor change ($p > 0.05$). Neither agitation nor suboptimal blood volume had significant effects ($p > 0.05$).

Conclusion. Specimens should ideally be tested within 12 hours or stored at 4°C to prevent degradation of the test variables, particularly white blood cell counts and platelet counts. The findings suggest that routine handling and transport conditions are appropriate, as agitation during transport and a decreased blood volume-to-anticoagulant ratio appear to have negligible effects. Incorporating measurement uncertainty benchmarks improves the distinction between genuine sample deterioration and assay variability, thereby enhancing diagnostic accuracy.

Keywords: complete blood count, specimen handling, storage time, specimen stability, laboratory best practices

DOI: 10.15386/mpr-2880

Manuscript received: 24.03.2025
Received in revised form: 02.09.2025
Accepted: 24.09.2025

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Introduction

The Complete Blood Count (CBC) is one of the most important and widely used diagnostic tools in clinical medicine, providing valuable hematological information. Values of multiple parameters in CBC are critical for the diagnosis and monitoring of many diseases. Physicians frequently rely on platelet counts, White Blood Cell (WBC) counts, and Red Blood Cell (RBC) counts in daily clinical practice to identify different diseases such as anemia, infections, or thrombocytopenia. By measuring these hematological parameters over time, they can also catch potential relapses, assess how fast a disease is progressing, and determine the best course of treatment.

Anemia is one of these cases, for which hemoglobin levels, Mean Corpuscular Volume (MCV), and the RBC count are among the significant criteria used for its diagnosis. Furthermore, platelet count is important for assessing thrombotic risk as well as diagnosing thrombocytopenia. Additionally, WBC count and differentials are usually used for infection measurement and inflammatory responses, as well as hematological malignancies.

However, the quality of the CBC test results can be affected by several variables, such as pre-analytical storage, transportation, handling, and laboratory procedures during analysis. These variables are all prone to measurement uncertainty and therefore lead to misdiagnoses and unnecessary testing. Failure to maintain sample stability, particularly through transportation, can contribute to hemolysis. This may falsely elevate the WBCs, obscure leukopenia and delaying the diagnosis of immune suppression or infection, and monitoring treatment with cytostatics.

Likewise, excessive agitation during transport can result in erroneous platelet counts, possibly contributing to the insufficient management of thrombocytopenia or unnecessary treatment [1]. Pre-analytical errors remain the most frequent cause of mistakes in hematology laboratories, and their prevention is a major focus of recent quality improvement programs [2].

Therefore, accurate CBC results are crucial for a precise laboratory diagnosis. Sample integrity requires control of proper temperature. If blood samples are stored at 4°C, the activity of such cells is slowed down so that their structure and function remain intact. At this temperature, enzymatic reactions are minimized, and therefore, it contributes to less hemolysis and more stable RBCs, WBCs, and platelets [3,4]. Conversely, for samples stored at ambient (~22°C) or elevated temperatures, cellular degradation can pose a risk, affecting their reliability for analysis. High temperatures, as observed in peripheral laboratories where ambient temperatures often exceed 26°C, can lead to practical deviations. These deviations can enhance several enzymatic reactions, resulting in hemolysis, swelling of RBCs, and platelet malfunction [3,4]. Many previous studies have shown that room temperature blood storage may increase MCV markedly, owing to RBC swelling, with consequences on probable misreading in anemia classification [4]. Freezing samples, however, can cause irreversible damage, primarily due to ice crystal formation, compromising the structural integrity of RBC and platelet membranes.

Physical damage from transport agitation, especially during vibrations in the range of 1–5 Hz, can lead to hemolysis and leukocyte degradation of blood samples [1]. Intracellular components released after hemolysis include hemoglobin and potassium, leading to the distortion of CBC measurements. For example, hemolysis can artificially increase WBC counts according to the number of fragmented cells, while at the same time, the hemoglobin and hematocrit values are affected. Excessive agitation may also disrupt platelet counts, causing micro-aggregates or clumps that are more easily misinterpreted by automated analyzers [1]. That is why we need to use adequate means of transport, both in terms of distance and agitation frequency, which are adapted to minimize these effects.

The quality of sample storage also influences CBC result reliability. Prolonged storage (beyond the 24-hour limit suggested by the World Health Organization) can significantly modify both leukocyte differentials and platelet morphology [4]. Extended storage at room temperature can induce apoptosis of neutrophils and promote the release of reactive oxygen species, both of which can result in artificially increased WBC counts. Storage appears especially harmful to platelets, which are known to undergo morphological changes. In clinical practice, this can lead to a falsely low platelet count with increased Mean Platelet Volume (MPV), which can complicate the diagnosis of thrombocytopenia and risk assessment for bleeding. [6] Furthermore, a recent comparative study showed that stability profiles may vary between hematology analyzers, highlighting the need for harmonized protocols across laboratories [7].

Inadequate blood volumes, particularly those that do not fill at least 50% of a tube's recommended capacity, can disturb the blood volume anticoagulant ratio and subsequent dilution or clotting of the sample. While the volume-to-anticoagulant ratio is critical, it is important to note that spray-dried K_2EDTA vacutainers do not introduce liquid dilution. However, under-filling may still affect test outcomes by altering the anticoagulant-to-blood surface interaction and promoting clot formation or platelet activation [8]. Excess anticoagulants may dilute the sample, influencing parameters such as hemoglobin concentration and hematocrit values, while insufficient anticoagulants can cause clot formation and render the sample unusable. For example, under-filled tubes cause hemoglobin miscalculations, and overfilled tubes increase clot formation, which affects platelet counts [8]. Standardized blood collection practices, such as proper vacutainer filling, are fundamental for consistent diagnostic results [9].

In addressing this challenge, it is relevant to know how different testing methods perform in various situations to achieve accurate CBC results. While much has been done studying these separate factors, we still have a poor understanding of their joint effects. The present study addresses this gap by systematically exploring the effects of temperature, agitation, storage time, blood volume anticoagulant ratio, and testing methods on CBC precision. By utilizing the mentioned variables of paired samples to propose a set of evidence-based guidance on optimal sample handling and processing conditions, the proposed framework will set a standard for advanced laboratory practices, which, if adopted, should significantly improve testing accuracy.

However, in many clinical settings, especially in primary care or peripheral laboratories, CBC samples are often transported over long distances, with transit times regularly exceeding 6 hours. Such real-world delays challenge the assumption of 8-hour processing windows and justify investigating the combined effects of pre-analytical stressors on CBC integrity, an area typically not covered in manufacturer validations.

Methods

Study design

This study utilizes a repeated-measures factorial design to investigate the impact of temperature, agitation, storage time, blood volume anticoagulant ratio, and testing methodology on CBC reliability. Venous blood was collected from eight adult volunteers into K_2EDTA tubes and processed under four handling conditions reflecting routine deviations: (i) optimal 2 mL fill, non-agitated; (ii) optimal 2 mL fill, agitated; (iii) sub-optimal 1 mL fill, non-agitated; and (iv) sub-optimal 1 mL fill, agitated. Samples were held at room temperature (22 ± 2°C); refrigerated holding (4°C) was used where indicated and is reported explicitly. The final analytic sample comprised 224 CBC runs. A consolidated flow diagram (Figure 1) displays branch-level counts.

Participant blood sample collection

Each of the eight participants provided four blood samples collected in 2 mL lavendertop BD Vacutainer® tubes (PET plastic) are spraycoated with K_2EDTA at 1.8 mg/mL of blood, equipped with a Hemogard™ closure, and are gammairradiated and sterile (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

A total of 224 CBCs are classified as follows:

Blood volume classification:

- Two samples were collected with optimal blood volume (2 ml) (as recommended by the manufacturer).
- Two samples were collected with suboptimal blood volume (1 ml) to allow the direct comparison of different volumes.

Agitation vs. non-agitation conditions:

Agitation: Simulated transport conditions with vibrations at 1–5 Hz (for 30 min) were compared to no agitation.

N.B.: Agitation was simulated using a programmable orbital shaker set to 1–5 Hz and 25–80 mm·s^{-2} RMS, based on real-world accelerometer measurements from our hospital's Tempus600® pneumatic tube system (median Root Mean Square acceleration (RMS): 32 mm·s^{-2}), and in agreement with the vibration thresholds reported by Cadamuro et al. [10] and Streichert et al. [11].

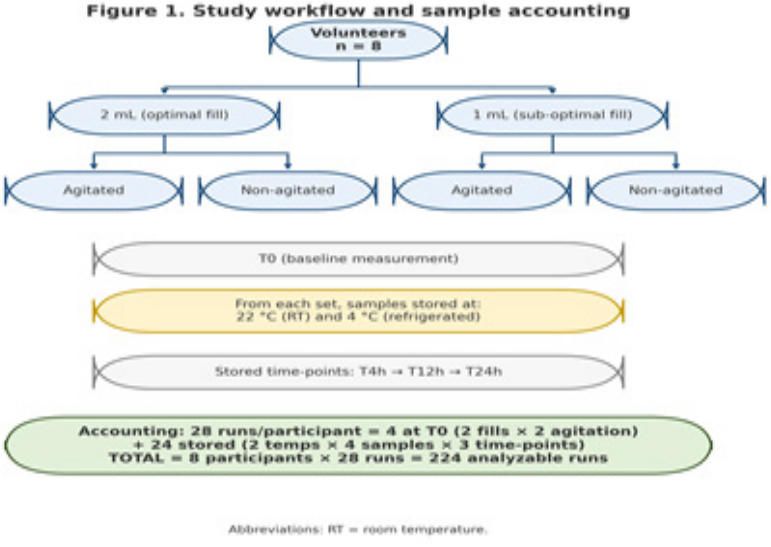

Figure 1. Study workflow and sample accounting. Eight volunteers provided paired blood samples under four pre-analytical conditions (2 mL optimal vs. 1 mL sub-optimal; agitated vs. non-agitated). Each sample was analyzed at baseline (T0) and after storage at T4h, T12h, and T24h. This design yielded 28 runs per participant and 224 runs in total (8 × 28).

• From the optimal blood volume group (2 ml):

o One sample was subjected to agitation during transport.

o One sample was not agitated (remained stable).

• From the suboptimal blood volume group (1 ml):

o One sample was subjected to agitation during transport.

o One sample was not agitated (remained stable).

*At this stage, as a basic state, a CBC run is performed for each participant's four samples.

Storage temperature conditions:

• Each group (Optimal-Agitated, Optimal-Non-Agitated, Suboptimal-Agitated, Suboptimal-Non-Agitated) was split into two storage conditions:

o One half was stored at Room Temperature (RT, ~22°C based on commonly reported ambient laboratory conditions in hematology laboratories and previous stability studies [12]).

o The other half was stored in a Refrigerator (4°C).

Time-based testing intervals:

• Every stored sample was analyzed at three specific time points to assess stability and parameter changes (a total of 24 CBC runs per participant):

o 4 hours, 12 hours, and 24 hours starting after applying the storage conditions

Recruitment of participants

To examine potential differences in hematological parameters, the research included eight healthy subjects, consisting of four males and four females, aged 25-45 years. Inclusion criteria: no smoking, fasting, and the absence of underlying medical disorders such as diabetes or hypertension were included. Ethical approval was obtained, and all participants provided written informed consent in line with the Declaration of Helsinki.

Samples collecting and handling

Trained phlebotomists used sterile methods to obtain blood samples that were carefully inverted eight to ten times after collection to provide an adequate anticoagulant mixing without adding agitation artifacts.

Laboratory analysis

An automated hematology analyzer (DIAGON D-Cell 60) was employed to measure RBC count, WBC count, and differentials, hemoglobin concentration, PCV, platelet count, MCV, and MPV. All samples were analyzed in one batch per time point, and every sample was repeated three times, while the averages were listed as the final result.

Three levels of control were performed before each stage of the CBC run (DIAGON D-Check D LOT:1X0901).

Following established CLSI norms, we assessed the analyzer's precision using internal quality control samples to ensure the accuracy of our CBC readings. With normal variance of 0.6% for hemoglobin, 1.0% for white blood cells, 0.8% for red blood cells, and 1.5% for platelets, the DIAGON D-Cell 60 demonstrated consistent findings under ideal lab circumstances. We computed the measurement error margins based on these measurements: ±1.2% for hemoglobin, ±1.5% for WBC and RBC, and ±2.0% for platelets (Table I). By comparing our observed changes in stored samples to these standards, we were able to assess if they exceeded the range of typical analytical variability.

Statistical analysis

The data were presented as mean ± standard deviation (SD) for every experimental condition. The major effects and interactions of temperature, agitation, storage time, blood volume anticoagulant ratio were examined using a four-way repeated-measures analysis of variance (ANOVA). Post-hoc pairwise comparisons using Bonferroni corrections were performed to analyze the significant differences between conditions. The effect sizes were calculated using partial eta squared (η^2).

Ethical considerations

The study was conducted following Good Clinical Practice (GCP) guidelines and approved by the institutional ethical review board. The participants were informed of the study's objectives, methods, and possible risks before any samples were collected, and they provided signed informed consent.

Broader implications

This study may have significant implications for clinical decision-making. For example, if specific pre-analytical factors are shown to significantly influence CBC, this would raise uncertainties about physicians' interpretation of results, which may affect therapeutic choices. The study will address these consequences, including recommendations for laboratory operations and the clinical interpretation of CBC results.

Table I. Analytical imprecision (Intra and Inter-Assay CV).

Parameter	CV_intra (%)	CV_inter (%)	Measurement Uncertainty (MU ±%)
Red Blood Cells (RBC)	0.8	1.0	±1.5
Hemoglobin (Hb)	0.6	0.9	±1.2
White Blood Cells (WBC)	1.0	1.3	±1.5
Platelets (PLT)	1.5	1.8	±2.0
Red cell distribution width (RDW)	1.4	1.5	±1.8
Mean platelet volume (MPV)	1.6	1.7	±1.5

Results

To establish benchmarks for interpreting stability, we first evaluated the analyzer's imprecision (Table 1). The DIAGON D-Cell 60 showed low intra- and inter-assay variation, with calculated measurement uncertainty (MU) margins of ±1.5% for RBC and WBC, ±1.2% for Hb, ±2.0% for platelets, ±1.8% for RDW, and ±1.5% for MPV. These MU thresholds were used as reference limits to judge whether observed changes exceeded expected analytical variability.

The results show a significant decrease in WBC count ($p < 0.001$), with the most pronounced reduction after 12 hours of storage. Storage duration had a strong effect size ($\eta^2 = 0.129$) (Figure 2). Although 4°C slowed WBC loss at earlier checkpoints, the 24-h drop was still larger at 22°C (−6.0 %) than at 4°C (−5.7 %) (Table II). Samples with optimal volumes showed slightly better stability than those with suboptimal volumes, but this difference was not significant ($p > 0.05$, $\eta^2 \approx 0$). Agitation did not affect WBC count ($p > 0.05$, $\eta^2 \approx 0$).

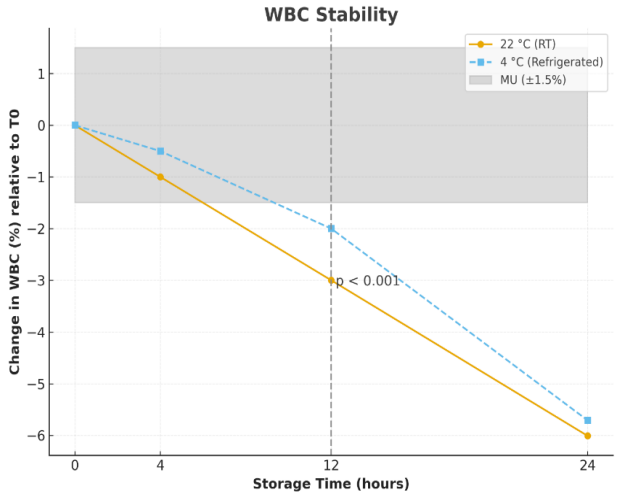

Figure 2. WBC stability. Change in WBC (%) relative to T0 at 22°C vs 4°C over 0–24 h. MU, ±1.5%. Decline became significant at 12 h ($p < 0.001$).

**Notes (apply to all figures): points depict mean % change vs. baseline (T0); Gray band = measurement uncertainty, negative values indicate decreases; storage times: 0, 4, 12, 24 h.*

According to hemoglobin levels and RBC counts, there were only minor fluctuations over time and temperature ($p > 0.05$). Nevertheless, after 24 h, both Hb and RBC values exceeded their analytical measurement-uncertainty limits at 22°C (Hb −2.28 % vs ±1.2 % MU; RBC −2.21 % vs ±1.5 % MU), while remaining within MU at 4°C (Table II), (Figures 3A and 3B). Storage duration had a moderate effect size ($\eta^2 = 0.08$), while the temperature effect was small and not significant ($\eta^2 = 0.025$). Agitation did not impact hemoglobin and RBCs ($p > 0.05$, $\eta^2 \approx 0$). Although not statistically significant ($p > 0.05$), optimal sample volumes showed slightly less variability compared to suboptimal samples.

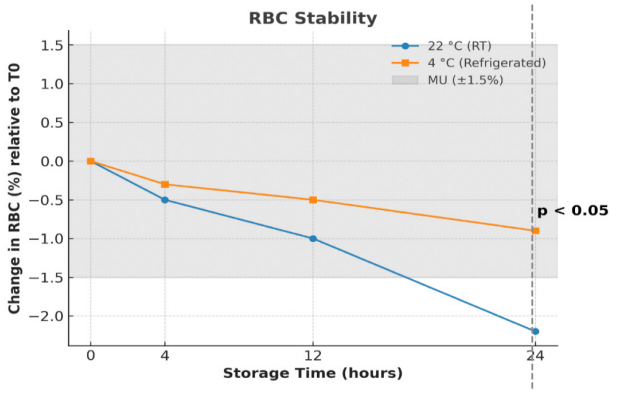

Figure 3A. RBC stability. Percent change vs. T0 with MU (±1.5%). At 22°C, the 24 h decrease exceeded MU ($p < 0.05$); at 4°C values remained within MU.

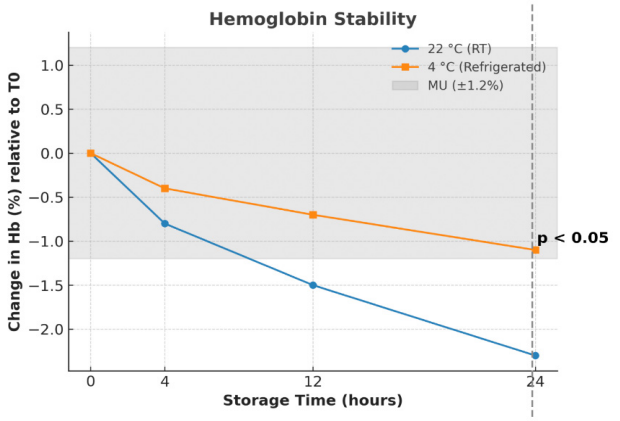

Figure 3B. Hemoglobin stability. Percent change vs T0 with MU (±1.2%). At 22°C, the 24 h decrease exceeded MU ($p < 0.05$); at 4°C, values remained within MU.

Table II. Mean percentage change from baseline in CBC parameters after 24 hours of storage at 22°C and 4°C compared with measurement uncertainty (MU) thresholds.

Parameter	Mean % Change at 22°C (24h)	Mean % Change at 4°C (24h)	Analytical MU from Table 1 (±%)
WBC	-6.01%	-5.71%	±1.5%
RBC	-2.21%	0.24%	±1.5%
Hb	-2.28%	0.52%	±1.2%
Platelets	-5.18%	-8.08%	±2.0%

At 22°C, MCV significantly increased over time ($p < 0.001$) (Figure 4A), and RDW showed comparable trends ($p < 0.001$), both suggesting RBC expansion with extended storage (Figure 4B). The effect of agitation on these measurements was minimal ($\eta^2 \approx 0$). While variations were relatively slight, optimal volumes yielded somewhat more stable values than suboptimal volumes.

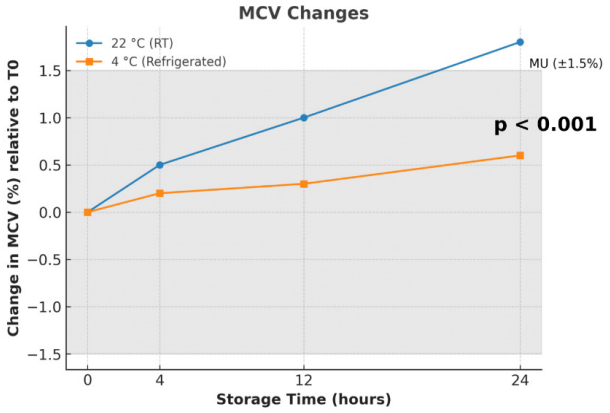

Figure 4A. MCV changes. Percent change vs T0 with MU (±1.5%). MCV rose at 22°C and was significant by 24 h ($p < 0.001$); minimal drift at 4°C.

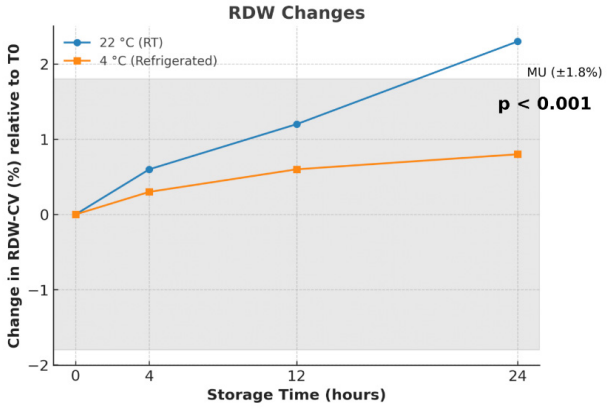

Figure 4B. RDW-CV changes. Percent change vs T0 with MU (±1.8%). RDW-CV increased at 22°C, significant by 24 h ($p < 0.001$); smaller rise at 4°C.

A significant decrease was detected in platelet count over time ($p < 0.001$), especially after 12 hours of storage. The effect size for storage time was large ($\eta^2 = 0.73$). Although storage at 4°C produced significantly tighter dispersion in platelet counts over the entire time-course ($p < 0.01$, $\eta^2 = 0.05$; Figure 5), the cumulative 24-h drift was still larger at 4°C (−8.1 %) than at 22°C (−5.2 %) (Table II). Agitation had no significant effect on platelet degradation ($p > 0.05$, $\eta^2 \approx 0$). Suboptimal (1 mL) samples exhibited slightly faster degradation and variability ($\eta^2 = 0.03$) compared to optimal (2 mL) samples, though no major differences were observed.

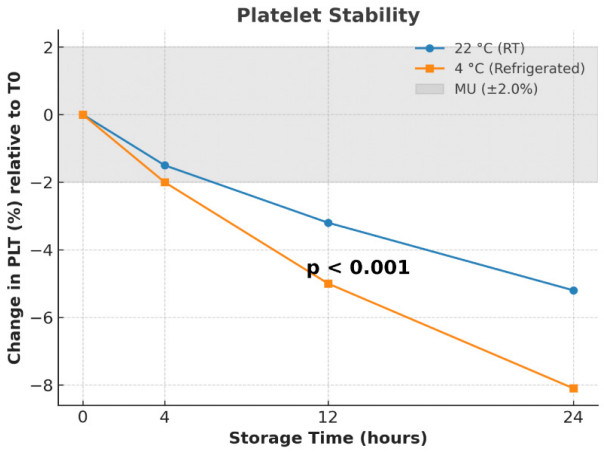

Figure 5. Platelet (PLT) stability. Percent change vs T0 with MU (±2.0%). Decline was significant at 12 h ($p < 0.001$) and progressed thereafter, greater at 4°C.

Comparison of observed changes with analytical CV thresholds

Some significant trends emerged when we compared the average changes in CBC parameters during a 24-hour storage period with the typical variability and measurement uncertainty of the analyzer (Table II). Platelet and white blood cell count that were kept at 4°C decreased by about 8.1 % and 5.71 %, respectively. These declines exceeded the typical expected range, demonstrating that sample deterioration cannot be completely avoided by refrigeration alone. However, hemoglobin remained within MU at 4°C (+ 0.52 %) but breached the ±1.2 % MU at 22°C (-2.28 %), whereas red-blood-cell counts breached the ±1.5 % limit at 22°C (−2.21 %) but not at 4°C (0.24 %). Approximately 2.8 % more hemoglobin decreased at room temperature, exceeding the ±1.2 % MU threshold. Overall, this comparison highlights how storage time and temperature can differently affect CBC results, emphasizing the need to process samples promptly and keep them properly cooled.

In addition, MPV remained stable over time ($\eta^2 = 0.03$) but exhibited some variations at 22°C ($p < 0.05$). Neither MPV nor PCV assessments were significantly influenced by agitation ($p > 0.05$, $\eta^2 \approx 0$). Significantly suboptimal volumes showed increased variability, most notably concerning PCV measurements.

Furthermore, the lymphocyte counts gradually declined after incubation ($p < 0.01$), and the decline at 22°C was greater than that at 4°C. Also, after 12 hours at room temperature, neutrophil counts were significantly reduced ($p < 0.001$), while neutrophils and lymphocytes stability remained unaffected by the agitation process ($\eta^2 \approx 0$). However, neutrophils were preserved better when the sample volume was at its optimal level.

Discussion

We investigated several preanalytical variables that could affect CBC results, particularly concerning storage time, temperature, agitation, and blood volume anticoagulant ratio. Our findings indicate a critical impact of storage parameters, especially time and temperature, on the CBC results, whereas agitation and blood volume anticoagulant ratio have minimal effects. These observations are consistent with preanalytical quality data and highlight the importance of appropriate handling and materials per a given standard [2,4,5,8,17].

Storage time was the defining element, as certain hematologic metrics changed meaningfully with prolonged preservation, particularly amongst more protractedly stored specimens. For instance, WBCs reduced with the progression of time ($p < 0.001$), with a reasonably sizable impact ($\eta^2 = 0.129$). Importantly, this decline was accentuated at room temperature, with a net 24-h fall of −6.0 % at 22°C versus −5.7 % at 4°C (Table II), underscoring the extra risk of ambient storage [2,4]. We also observed a statistically significant increase with time ($p < 0.05$) in both MCV and RDW, which was mostly attributed to changes in cell membrane permeability. Notably, both RBC and hemoglobin drifted just beyond their MU limits after 24 h at 22°C (−2.2 % and −2.3 %, respectively), indicating that prolonged ambient storage can mask mild anemia or blood-loss trends that lie near decision thresholds. In parallel, MPV showed a modest but significant rise at room temperature, echoing earlier reports of storage-related platelet swelling. Furthermore, the significant reduction in platelet counts over time ($p < 0.001$, $\eta^2 = 0.73$) suggests that the length of storage is the main factor influencing platelet viability, likely resulting from metabolic depletion and apoptosis [13]. Although dispersion was tighter at 4°C, the absolute 24-h loss was larger under refrigeration (−8 %) than at room temperature (−5 %), indicating cold-induced platelet injury (Table II). Refrigeration narrows the short-term spread in platelet counts, but it also speeds up cold-induced membrane damage, so the total platelet loss after 24 h ends up larger despite the tighter CV. Additionally, lymphocyte counts exhibited a decline over time ($p < 0.01$), reaching their peak at 22°C instead of 4°C. Similarly, neutrophil counts decreased significantly ($p < 0.001$) after 12 hours at room temperature, indicating that prolonged storage conditions may lead to increased activation and eventual cell death [14]. Simultaneously, other parameters held relatively consistent irrespective of storage length. While the evaluation of time effects is essential, the interplay of various influences merits additional exploration to gain a deeper understanding.

However, some earlier studies reported minimal or no significant changes under specific conditions. Xu et al. [18] observed that under-filled K_2EDTA tubes produced comparable CBC results to standard volumes when measured within one hour, suggesting robustness to sample volume. Similarly, Unalli & Özarda [19] found that RBC, platelet, and hemoglobin remained stable up to 48 h, with only modest changes in MCV and WBC at room temperature. Kakkar et al. [20] also noted acceptable stability of most indices for up to 24 h, particularly under refrigeration. These findings emphasize that while some studies found limited changes, our results demonstrate that when multiple preanalytical stressors are combined (time, temperature, agitation, and suboptimal volume), changes can exceed analytical thresholds, reflecting real-world laboratory conditions.

This is important in timely sample processing, which is well established in major recommendations such as WHO [5] and CLSI GP41 [8]. In normal laboratory practice, it is essential to define strict limits for acceptable storage time and temperature, following best quality control practices, to prevent sample degradation [3,4,8].

While there are concerns that vibration or movement during transport could affect hematological parameters, our results aligned with previous studies showing a minimal impact [1]. Whether samples were gently agitated or transported under standard clinical conditions did not result in statistically significant changes in WBC, RBC, or platelet counts. This finding may help minimize concerns regarding routine transport conditions, but laboratories should still follow consistent protocols and temperature controls to ensure uniformity [8,17].

Comparing insufficient blood samples to anticoagulant ratio (1 mL) to standard amounts (2 mL) did not substantially affect the majority of CBC indices. Thus, hemoglobin, RBC, and WBC levels stayed constant ($p > 0.05$). However, platelet counts from underfilled tubes had a slightly broader distribution ($\eta^2 = 0.03$), potentially a sign of platelet clotting or degradation due to insufficient blood volume. which is consistent with Becan McBride et al. [8] findings, discussing the alterations in hemostatic parameters in under-filled tubes. It also aligns with the established phlebotomy standards from CLSI [9] and the CAP Q-Probes studies [23], both of which highlight the necessity of the following recommended fill volumes to maintain optimal blood-to-anticoagulant ratios.

Therefore, appropriate sample handling management is extremely relevant, as any preanalytical factor has important clinical and economic repercussions. Karcher et al. found that reducing laboratory errors can lead to fewer redraws and better diagnostic precision [22]. Their findings, along with those of several CAP Q-Probes studies, indicate that specimen handling errors (like improper temperature or under-filling) contribute significantly to increased costs and results delay. Reducing false positive results can also reduce the costs and time associated with repeat draws. Hence, in many cases, proper storage conditions can help lower these unnecessary costs. On a macro level, enhancing CBC testing consistency can eventually help hospitals manage resources better while

further improving patient care, according to WHO [5] and CLSI guidelines [8].

Routine practice aims to test blood within eight hours and keeps QC CV < 10 % to limit pre-analytical error yet batching and transport in outpatient or peripheral networks often push storage well beyond this window. Our study mimicked those real-world delays by combining temperature shifts, agitation, and suboptimal fill volume factors usually excluded from standard validation. We show that storage > 12 h, particularly at room temperature, produces clinically significant shifts in platelet and WBC counts. Applying analytical-imprecision limits enables labs to distinguish true degradation from normal assay noise and set evidence-based storage rules [5,9].

Conclusion

The present study notes the considerable influence of storage conditions (time and temperature) on the stability of hematological parameters after blood storage, whereas agitation and blood volume have only a minimal influence. White-blood-cell counts declined more at room temperature, platelet counts fell more under refrigeration, and red-blood-cell counts exceeded MU limits only at 22°C, while hemoglobin remained within limits at 4°C but exceeded the ± 1.2 % MU threshold after 24 h at 22°C. Nevertheless, it is advisable to adhere to the suggested handling protocols since even little variations in platelet counts due to underfilled volumes and the tendency of WBCs to deteriorate during storage might have negative effects. Suboptimal blood volumes and agitation during transportation had no impact on the results. Based on these results, the current best practices include cooling for extended storage and testing within 12 hours if possible. According to national and international organizations that regulate laboratory medicine, standardized and evidence-based preanalytical methods are crucial for enhancing clinical decision-making, lowering errors, and reducing expenses [15,16,21–24]. Additionally, applying measurement uncertainty benchmarks enables laboratories to more accurately interpret changes due to sample degradation versus analytical variability, ultimately enhancing diagnostic confidence and laboratory quality control.

Recommendations

Based on our results and to optimize CBC sample stability, we focus on the following practical recommendations:

1. White blood cells (WBC)

Test samples within 12 hours, particularly if stored at room temperature (22°C). Refrigerate samples if testing may exceed 8 h; however, counts can still fall ~6 % after 24 h, so analyze within 12 h wherever possible.

2. Platelet count

Collected samples cannot be left at room temperature for more than 12 hours. If there is a delay, samples must be refrigerated but be aware that counts can drop ~8 % by 24 h even when refrigerated; prompt (< 12 h) testing is safest. Ensure that tubes are filled to at least 2 mL to help reduce fluctuations in platelet counts.

3. Red blood cells (RBC) and hemoglobin

Hemoglobin remains within MU at 4°C but breaches the ± 1.2 % MU threshold after 24 h at 22°C; therefore, test within 12 h or keep samples refrigerated. Red-blood-cell counts stay acceptable for 24 h if refrigerated but may exceed MU at room temperature (−2.2 %); test sooner whenever precise RBC values are critical.

4. MCV and RDW

Prolonged storage at 22°C causes erythrocyte swelling after ≈12 h, leading to significant rises in MCV and/or RDW that can misclassify anemia phenotypes. Refrigeration at 4°C largely prevents these shifts and preserves index stability.

5. MPV

MPV showed minor but significant increases at room temperature; therefore, if accurate MPV is critical, be cautious with samples stored at room temperature for prolonged times.

6. Packed cell volume (PCV)

Test for PCV within 12 h if kept at 22°C or refrigerate and test within 24 h.

7. Transport agitation

Routine transport does not appear to harm CBC results. No special measures are needed for mild agitation.

Limitations

Addressing some of the study's limitations is just as important as acknowledging its advantages. Although the study was designed as a pilot with a limited sample size (n = 8), limiting the generalizability of the results, the paired-sample design and repeated-measures technique involved a total of 224 CBC runs, supporting the study's statistical power.

Acknowledgments

The authors would like to express their gratitude to all the participants who contributed to this study. We are immensely grateful to the laboratory staff who helped us acquire and examine our test materials.

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