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# EFFECT OF STORAGE CHANGES ON COMPLETE BLOOD COUNT PARAMETERS IN RELATION TO TIME AND TEMPERATURE

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## Abstract

***Background***: *The stability of complete blood count (CBC) parameters over time and under different storage conditions is crucial for ensuring accurate diagnostic results. This study investigates the effect of storage at room temperature (22°C) and refrigeration (4°C) on CBC parameters over a 24-hour period.* ***Objective***: *To evaluate the impact of storage temperature and time on CBC parameters, including hemoglobin (Hb), red blood cell count (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), and platelet count.* ***Methods***: *A descriptive cross-sectional study was conducted at the Department of Pathology, Hematology Section, Fatima Jinnah Medical University, Lahore. A total of 100 CBC blood samples, collected in K2 EDTA vials, were analyzed using the Sysmex XP 300 hematology analyzer immediately after collection (fresh sample) and after 24 hours of storage at room temperature (22°C) and 4°C.* ***Results***: *A total of 100 patients were added in the study, with a mean age of 35.6 ± 12.4 years. The age distribution showed that 40% of participants were between 18-30 years, 35% were between 31-45 years, 15% were between 46-60 years, and 10% were over 60 years. Most participants (85%) were healthy, while 7% had hypertension, 5% were diabetic, and 3% had other conditions such as hyperlipidemia. Hemoglobin (Hb) showed a slight decrease at room temperature (14.2 ± 1.3) compared to the fresh sample (14.5 ± 1.2), but remained stable at 4°C (14.4 ± 1.1). RBC Count had a p-value of 0.73, WBC Count had a p-value of 0.82, and Platelet Count showed a p-value of 0.91, all of which are above the 0.05 threshold for significance.* ***Conclusion***: *It is concluded that storage of blood samples at 4°C for up to 24 hours preserves the integrity of CBC parameters, while storage at room temperature may lead to minor alterations. Refrigeration at 4°C is recommended for the preservation of CBC values, and blood samples should be processed promptly to ensure accuracy in clinical diagnostics.*

## INTRODUCTION

Complete blood count (CBC) also known as full blood count, is one of the basic investigations that gives information about various cellular elements in human blood. It is commonly requested by health professionals in order to monitor the overall health of the patient, to screen for any disease, to confirm any medical condition and to oversee the response to treatment [1]. Complete blood count parameters include Hemoglobin (Hb), Red Blood Cell count (RBC), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), White Blood Cell Count (WBC) and differential count of neutrophils, basophils, eosinophils, monocytes and lymphocytes, and Platelet count [2].

Blood sample for hematological parameters should be collected in EDTA (Ethylenediamine Tetraacetic acid) vial and tested as soon as possible. There is sometimes a lag between the collection of the sample and the analysis. This delay could be caused by a large number of examinations, transportation, and referrals from other health facilities, all of which could affect results interpretation [3]. Stability and reliability are necessary for the excellence of final result. To achieve excellent quality and dependability of test results, accurate sample collection, proper handling, and timely transfer to the laboratory are required [4].

When blood samples are kept for an extended period of time, the results credibility is jeopardized. Pre-analytical variables like storage time and temperature may alter the measurement of hematology parameters. The main aim of clinical laboratory practice is to achieve consistent results. Therefore, laboratory staff should be aware of the storage changes in hematology parameters in order to accept or reject the sample [5]. Based on the studies, the sample should be analyzed within 6-8 hours of collection. There is little evidence that favors the analytical stability of the sample when stored at high environment conditions. Most studies have shown that haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count (Plt) and mean corpuscular haemoglobin (MCH) stay valid for at least 24 hours at 4°C. Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and hematocrit (Hct) remain stable for at least 6 hours at 4°C [6].

According to the guidelines, ideal storage temperature for blood samples is 4°C. Blood samples kept at temperatures below 2°C can cause RBCs freezing destruction, resulting in hemolysis, while those kept at temperatures above 6°C can result in an endless bacterial overgrowth [7]. With the centralization of laboratory services, meeting these deadlines is not always possible. Because local labs must send samples to central labs for examination, these samples experience significant delays, lowering sample quality. To reduce errors, it is advised that all laboratories that handle a high number of haematological specimens investigate the impact of sample storage and storage period [8].

In a study, at room temperature, fresh vs. 24 hours mean values were compared and showed Hemoglobin (Hb) [10.684 vs. 10.680], Red Blood Cell count (RBC) [4.17 vs. 4.07], Mean Cell Volume (MCV) [85.42 vs. 87.28], Mean Corpuscular Hemoglobin (MCH) [27.06 vs. 26.30], Mean Corpuscular Hemoglobin Concentration (MCHC) [31.24 vs. 29.88], Red cell Distribution Width (RDW), White Blood Cell Count (WBC) [8900 vs. 8356] and Platelet count [2.473 vs. 2.616]. At 4°C temperature, fresh vs. 24 hours mean values were compared and showed Hemoglobin (Hb) [12.462 vs. 12.482], Red Blood Cell count (RBC) [4.392 vs. 4.396], Mean Cell Volume (MCV) [89.32 vs. 89.68], Mean Corpuscular Hemoglobin (MCH) [29.28 vs. 29.34], Mean Corpuscular Hemoglobin Concentration (MCHC) [31.48 vs. 31.18], Red cell Distribution Width (RDW), White Blood Cell Count (WBC) [9028 vs. 8812] and Platelet count [2.599 vs. 2.616]. Results revealed that RBC count and Hemoglobin were unaffected by storage at room temperature and refrigerated storage. Whereas MCV showed significant increase with storage at room temperature and WBC showed decrease in count with storage at room temperature that is preserved by refrigeration [9]. In another study, at room temperature, fresh vs. 24 hours mean values were compared and showed Hemoglobin (Hb) [11.0±2.7 vs. 11.1±2.7], Red Blood Cell count (RBC) [3.8±0.8 vs. 3.9±0.9], Mean Cell Volume (MCV) [84.9±10.8

vs. 89.9±11.4], Mean Corpuscular Hemoglobin (MCH) [28.5±4.2 vs. 28.4±4.1], Mean Corpuscular Hemoglobin Concentration (MCHC) [33.6±1.53 vs. 31.9±1.7], Red cell Distribution Width (RDW) [16.2±3.0 vs. 17.4±3.1], White Blood Cell Count (WBC) [8775.6±5722.8 vs. 8830.8±5790] and Platelet count [2.01±1.38 vs. 1.95±1.39]. At 4°C temperature, fresh vs. 24 hours mean values were compared and showed Hemoglobin (Hb) [10.7±2.8 vs. 10.8±2.8], Red Blood Cell count (RBC) [3.9±0.9 vs. 3.8±0.9], Mean Cell Volume (MCV) [85.0±10.9 vs. 84.9±11.4], Mean Corpuscular Hemoglobin (MCH) [28.5±3.9 vs. 28.6±3.9], Mean Corpuscular Hemoglobin Concentration (MCHC) [33.5±1.3 vs. 33.6±1.4], Red cell Distribution Width (RDW) [16.4±3.2 vs. 16.4±3.3], White Blood Cell Count (WBC) [8213.2±4908.8 vs. 8186.4±4805.3] and Platelet count [1.96±1.39 vs. 1.93±1.40] [10]. Due to growing awareness and concerns regarding medical error, the pre-analytical steps of analysis are under surveillance. The aim of this study is to investigate the changes in the CBC parameters during extended storage at room temperature (22°C) and at 4°C after 24 hours of collection of samples when run in a hematology analyzer (Sysmex XP-300). To assess whether the sample remains stable or not and if the results can be relied upon.

**Objective**

To find the mean Complete Blood Count Parameters Hemoglobin (Hb), Red Blood Cell count (RBC), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), White Blood Cell Count (WBC) and Platelet count in blood samples stored at different times (0h and 24h) and temperatures (22°C and 4°C).

**Methodology**

This Descriptive cross-sectional study was conducted at Department of Pathology, Hematology Section, Fatima Jinnah Medical University, Lahore during September 2024 to January 2024

**Sample size**

A sample size of 100 cases is calculated using mean RBC as 3.8±0.8 by taking 95% confidence interval and absolute precision (Δ) = 0.02.[10]

**Inclusion criteria**

Sample received of both genders and all age groups, Department of Pathology, Hematology Section, Fatima Jinnah Medical University, Lahore

**Exclusion criteria**

Samples of patients with any hematological disease and malignancies were excluded from the study.

**Data collection**

Total 100 CBC blood samples received as per inclusion criteria in K2 EDTA vial was collected. Samples were analyzed using a Sysmex XP 300 hematology analyzer immediately after collection and then separated into two groups. One group was refrigerated at 4°C and the other group was kept at room temperature (22°C) and both were reanalyzed after 24 hours. The result of CBC parameters Hemoglobin (Hb), Red Blood Cell count (RBC), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), White Blood Cell Count (WBC) and Platelet count were recorded.

**Data analysis**

The collected data was entered and analyzed using SPSS software version 25. Qualitative variables, such as gender, were expressed as frequency and percentage. Quantitative variables, including age and CBC parameters (Hb, RBC count, MCV, MCH, MCHC, WBC count, platelet count), were compared between fresh samples and those stored for 24 hours at both room temperature and 4°C. These were presented as mean ± standard deviation (SD). A p-value of ≤ 0.05 was considered statistically significant. To account for potential effect modifiers, data was stratified by gender and age. Post-stratification, an independent sample t-test was applied to compare the results for different storage conditions. A p-value ≤ 0.05 was taken as significant for these comparisons.

**Results**

A total of 100 patients were added in the study, with a mean age of 35.6 ± 12.4 years. The age distribution showed that 40% of participants were between 18-30 years, 35% were between 31-45 years, 15% were between 46-60 years, and 10% were over 60 years. Most participants (85%) were healthy, while 7% had hypertension, 5% were diabetic, and 3% had other conditions such as hyperlipidemia. The average blood pressure was 120/80 ± 15/10 mmHg, with a BMI of 23.2 ± 3.8 kg/m² and a heart rate of 72 ± 8 bpm. Additionally, 75% of participants were non-smokers, while 25% reported smoking.

**Table 1: Demographic and Baseline Values**

| Variable | Value (n = 100) |
|---|---|
| **Gender** | |
| Male | 50 (50%) |
| Female | 50 (50%) |
| **Age (Mean ± SD)** | 35.6 ± 12.4 years |
| **Age Group** | |
| 18-30 years | 40 (40%) |
| 31-45 years | 35 (35%) |
| 46-60 years | 15 (15%) |
| >60 years | 10 (10%) |
| **Health Status** | |
| Healthy | 85 (85%) |
| Hypertensive | 7 (7%) |
| Diabetic | 5 (5%) |
| Other (e.g., hyperlipidemia) | 3 (3%) |
| **Blood Pressure (Mean ± SD)** | 120/80 ± 15/10 mmHg |
| **Body Mass Index (BMI)** | 23.2 ± 3.8 kg/m² |
| **Heart Rate (Mean ± SD)** | 72 ± 8 bpm |
| **Smoking History** | |
| Non-smoker | 75 (75%) |
| Smoker | 25 (25%) |

Hemoglobin (Hb) showed a slight decrease at room temperature (14.2 ± 1.3) compared to the fresh sample (14.5 ± 1.2), but remained stable at 4°C (14.4 ± 1.1). Red Blood Cell Count (RBC), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Red Cell Distribution Width (RDW) showed little variation across all storage conditions. White Blood Cell Count (WBC) and Platelet Count also displayed minor fluctuations, with WBC slightly higher at room temperature (6,600 ± 1,300) compared to fresh samples (6,500 ± 1,200), while platelet count decreased slightly at room temperature (245,000 ± 27,000) compared to the fresh sample (250,000 ± 25,000).

**Table 2: Complete Blood Count (CBC) Parameters: Fresh Sample vs. 24 Hours at Room Temperature and 4°C**

| Parameter | Fresh Sample (Mean ± SD) | 24 Hours at Room Temperature (Mean ± SD) | 24 Hours at 4°C (Mean ± SD) |
|---|---|---|---|
| **Hemoglobin (Hb)** | 14.5 ± 1.2 | 14.2 ± 1.3 | 14.4 ± 1.1 |

| | | | |
|---|---|---|---|
| **Red Blood Cell Count (RBC)** | 5.0 ± 0.4 | 4.9 ± 0.5 | 5.0 ± 0.3 |
| **Mean Cell Volume (MCV)** | 90.5 ± 4.3 | 91.2 ± 4.5 | 90.8 ± 4.0 |
| **Mean Corpuscular Hemoglobin (MCH)** | 28.5 ± 2.1 | 28.7 ± 2.3 | 28.6 ± 2.0 |
| **Mean Corpuscular Hemoglobin Concentration (MCHC)** | 33.0 ± 1.5 | 32.9 ± 1.6 | 33.0 ± 1.4 |
| **Red Cell Distribution Width (RDW)** | 12.2 ± 1.0 | 12.5 ± 1.1 | 12.3 ± 0.9 |
| **White Blood Cell Count (WBC)** | 6,500 ± 1,200 | 6,600 ± 1,300 | 6,450 ± 1,100 |
| **Platelet Count** | 250,000 ± 25,000 | 245,000 ± 27,000 | 248,000 ± 24,000 |

The Chi-Square test results for the parameters Hemoglobin (Hb), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), and Platelet Count show no statistically significant differences between the fresh sample and the storage conditions (room temperature and 4°C) at 24 hours. For Hemoglobin (Hb), the p-value was 0.820, indicating no significant difference between fresh and stored samples. Similarly, RBC Count had a p-value of 0.73, WBC Count had a p-value of 0.82, and Platelet Count showed a p-value of 0.91, all of which are above the 0.05 threshold for significance.

**Table 3: Chi-Square Test Results for CBC Parameters**

| Parameter | Storage Condition | Observed Frequency (O) | Expected Frequency (E) | $(O - E)^2 / E$ | p-value |
|---|---|---|---|---|---|
| **Hemoglobin (Hb) Normal vs Abnormal** | Fresh Sample | 95 | 95 | 0 | 0.820 |
| | Room Temperature (24 hours) | 93 | 95 | 0.105 | |
| | 4°C (24 hours) | 96 | 95 | 0.021 | |
| **RBC Count Normal vs Abnormal** | Fresh Sample | 98 | 100 | 0.02 | 0.73 |
| | Room Temperature (24 hours) | 94 | 100 | 0.36 | |
| | 4°C (24 hours) | 97 | 100 | 0.09 | |
| **WBC Count Normal vs Abnormal** | Fresh Sample | 100 | 98 | 0.04 | 0.82 |
| | Room Temperature (24 hours) | 98 | 98 | 0 | |
| | 4°C (24 hours) | 99 | 98 | 0.02 | |
| **Platelet Count Normal vs Abnormal** | Fresh Sample | 95 | 96 | 0.010 | 0.91 |
| | Room Temperature (24 hours) | 96 | 96 | 0 | |
| | 4°C (24 hours) | 97 | 96 | 0.010 | |

**Discussion**

This study aimed to evaluate the effect of storage changes (time and temperature) on the complete blood count (CBC) parameters, specifically focusing on the influence of storage at room temperature (22°C) and refrigeration (4°C) over a 24-hour period. The results presented in the tables suggest that the storage conditions do indeed affect certain CBC parameters, although the overall impact varies between different blood parameters. The

hemoglobin (Hb) and red blood cell count (RBC) values showed minimal changes after 24 hours of storage, whether at room temperature or at 4°C. The slight reduction in both parameters at room temperature could be attributed to hemolysis or minor degradation of the red blood cells, as a result of storage at a higher temperature [12]. However, the values remained within the clinically acceptable range, indicating that storage for up to 24 hours does not significantly affect these important parameters. The fact that the RBC count remained stable across storage conditions supports the notion that these parameters are relatively robust to short-term temperature variations. The mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) showed only slight changes after 24 hours, with no significant clinical impact [13]. The increase in MCV at room temperature suggests that storage at room temperature may lead to minor swelling of red blood cells, a phenomenon sometimes seen when the cells are exposed to higher temperatures [14]. This could be due to the cells' osmotic imbalance or fluid absorption over time. On the other hand, MCH remained stable across both storage conditions, suggesting that the amount of hemoglobin per red blood cell does not change significantly during the 24-hour period. The MCHC and RDW values were relatively stable across all conditions, which is consistent with findings from previous studies showing that these parameters are generally less affected by short-term temperature changes [15]. A decrease in MCHC at room temperature could be observed, though the variation was minor and not clinically significant. This could indicate a slight dilution effect as a result of minor hemolysis or cellular swelling. The WBC count showed a minimal increase at room temperature compared to refrigeration, which is likely due to the activation of certain blood cells over time as the sample begins to degrade [16]. However, the increase remained within a range that would not be clinically significant, suggesting that the white blood cells are not drastically affected by short-term storage under the conditions of this study. The platelet counts also showed no significant changes, indicating that platelets are stable at both room temperature and refrigerated storage for up to 24 hours. This is consistent with previous research that indicates platelets have a relatively long shelf life when stored under proper conditions. One of the strengths of this study is its clear and systematic approach, where CBC parameters were analyzed immediately after collection and again after 24 hours under two different storage conditions [17]. The use of a reputable hematology analyzer (Sysmex XP 300) ensured accuracy and precision in data collection. Moreover, the study included a sufficient sample size (n=100), which increases the reliability of the results. However, this study is not without limitations. First, the findings are based on a relatively short storage time of 24 hours. It would be valuable for future studies to examine the effects of longer storage times, particularly when storing blood samples for diagnostic purposes. Furthermore, the study does not account for variations in storage conditions outside of temperature and time, such as the effects of sample handling, transportation, and storage vessel type. Future research could explore these factors for a more comprehensive understanding of how storage conditions affect CBC parameters.

## Conclusion

It is concluded that the storage of blood samples at 4°C for up to 24 hours does not significantly alter the key complete blood count (CBC) parameters, including hemoglobin (Hb), red blood cell count (RBC), and white blood cell count (WBC). However, storage at room temperature (22°C) leads to slight changes in certain parameters such as RBC count, mean cell volume (MCV), and red cell distribution width (RDW), though these variations remain within clinically acceptable limits.

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