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# Effects of storage of blood sample at room temperature on measurement of red blood cell parameters

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**Background:** Accurate measurement of hematologic analytes is essential for physicians to establish correct diagnoses, plan of patient's treatment, evaluate treatment response and monitor the progression of disease over time. For the most accurate hematological results, blood specimens should be analyzed as soon as possible after collection. This study aimed to determine the effect of storage durations of blood samples stored at room temperature on the measurement of red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), red cell indices (MCV, MCH, MCHC) and red cell distribution width-Coefficient of variation (RDW-CV).

**Methods:** A cross sectional study was conducted between 2019 to 2020. Fifty healthy students (male and female) were randomly selected according to predefined inclusion and exclusion criteria. Blood samples were collected into EDTA tubes. RBCs parameters were measured immediately after collection to obtain baseline values, then stored at room temperature (24°C) and further measurements were obtained at 6, 12 and 24 hours after collection. Analysis was performed using fully automated hematology analyzer (sysmex XP-300).

**Results:** Data were analyzed using Statistical Package for Social Sciences (SPSS) computer program (version 20). P-value <0.05 was considered statistically significant. The study proved that RBC's count, Hb and MCH were least affected by storage at 24°C, showed stability after 24 hours (P= 0.943, 0.999, 0.75 respectively). In contrast, MCV showed a significant increase after 6 hours (P= 0.002), and PCV increased significantly after 12 hours (P< 0.001). A highly significant decrease was observed in MCHC after 12 hours (P< 0.001). While RDW-CV, revealed a highly significant increase after 12 hours (P < 0.001).

**Conclusion:** The study concluded that some RBCs parameters are changed with storage at room temperature. Therefore, it is recommended that RBCs parameters should be measured immediately after blood sample collection.

***Keywords:*** *RBCs count, Hb, PCV, MCV, MCH, MCHC, RDW-CV and Room temperature*

## Introduction

Accurate measurement of hematological analytes is essential to the physicians to make correct diagnoses, plan of patient's treatment, evaluate the response to therapy and monitor the course of the disease over time (Schapkaitz & Pillay, 2015). For the most reliable hematological results, blood specimens should be analyzed as soon as possible after

collection (Hill et al., 2009). Factors in the pre-analytical phase including specimen collection, handling and storage are critical components affecting clinical laboratory results (Cheesbrough, 2006). Samples may be drawn and then transported at ambient temperature to another laboratory; delayed hematological analysis may yielded doubtful or often invalid results. Complete Blood Count (CBC) is one of the most basic tests performed on the peripheral blood. It is comparatively inexpensive yet serves as a powerful diagnostic tool for a wide range of conditions (Lokwani, 2013). Even in routine health checkups of apparently healthy individuals, CBC is often the first step to detecting underlying illness, since this test has become easy and quick to perform (Mahmoodi et al., 2006), it provides a myriad of valuable information about the blood and to some extent the bone marrow the factory responsible for producing all blood element. CBC serve as a window into the functional status of the bone marrow and provides both direct and indirect evidence of the health and disease status of various body systems. This is achieved through a series of measurements that evaluate the composition and concentration of different blood cell components, including RBCs count, hemoglobin level, hematocrit, red blood cell indices and red cell disruption width (Lokwani, 2013). Red blood cell (RBC) count is expressed as millions of cells per liter of blood. This parameter is clinically important as it reflects the oxygen-carrying capacity of the blood. Assessment of the RBC count is essential for the detection of anemia and for evaluating the status of normal erythropoiesis (Lokwani, 2013). Hemoglobin is the vital oxygen-carrying component of red blood cells, and it is essential for sustaining life, every organ in the human body depends on oxygenation for growth and function, a process ultimately controlled by hemoglobin (Lokwani, 2013). Packed cell volume (PCV), also referred to as hematocrit, represents the proportion of whole blood occupied by red cells. It is often used to screen for anemia when direct hemoglobin measurement is not possible (Cheesbrough, 2006). The red blood cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), provide important information regarding the size and hemoglobin content of red blood cells (Ciesla, 2012). Red cell distribution width (RDW) is a quantitative measure or numerical expression of anisocytosis. Anisocytosis of RBCs is a variation of cell size in a given population of cells or excessive heterogeneity on smear or visual equivalent of increased CV (coefficient of variation) (Lokwani, 2013). Sample stability is defined as the ability of a sample to retain the initial value of a measured quantity for a defined period within specific limits, when stored under controlled conditions. Clinically, sample stability refers to the use of the reference range as a criterion, considering an analyte stable if the difference between the measured value and the initial value does not exceed 1/12 of the method's reference range (Imeri et al., 2008). Blood specimens collected in primary care often require transportation to central laboratories for analysis. To preserve the original concentration of components, separation of cells from serum or plasma combined with cooling or freezing during transport is commonly employed, since hematological parameters are generally more stable at lower temperatures. Time is a critical factor between sample collection and analysis, and it is widely accepted that blood specimens should be assayed with minimal delay. Typically, hematological parameters are measured from EDTA-anticoagulated whole blood shortly after collection (Schapkaitz & Pillay, 2015). However, various changes occur in anticoagulated blood stored at room temperature, and these changes progress more rapidly at higher ambient temperatures, regardless of the anticoagulant used (Bain et al., 2011). The present study therefore examines the changes in RBCs count, hemoglobin concentration, PCV, RBC indices and RDW-CV and evaluates the validity of results obtained from samples stored at room temperature when cannot be analyzed in a timely manner.

## Materials and Methods

This cross-sectional study was conducted at the Faculty of Medical Laboratory Sciences, University of Gezira, located in Wad Madani City, Gezira State in central Sudan, between 2019 to 2020. A total of 50 students free from illness and in normal health were randomly selected according to predefined inclusion and exclusion criteria. Any history of chronic or acute illness, using of cigarettes or alcohol, use of drugs or vitamins that change RBCs parameters, history of blood loss or surgical operation, were excluded. Ethical clearance was obtained from the Research Ethical Committee (REC) of the faculty of medicine, university of Gezira. Verbal consent was taken from each study subject before sample collection. Venous blood was withdrawn from the antecubital vein and sample collection was done by aseptic condition. The tourniquet was released as soon as the blood begins to flow into the syringe. A total of 3 ml of venous blood was drawn and released into EDTA containers tube. The blood was mixed with anticoagulant by gently inverting the tube three times and the tube was labeled (Bain et al., 2011). The samples were then analyzed using a fully automated hematology analyzer (sysmex XP-300). Sysmex XP-300 is a compact instrument, perform a reliable analysis of 20 parameters using Direct Current "DC" Resistance principle and display results in 3 histograms on the LCD screen. Also, the analysis data can be printed on the internal/external printer. Laboratory analysis was performed immediately after collection to obtain baseline value. The samples were subsequently stored at room temperature (24 °C), further measurements were obtained after 6, 12 and 24 hours from collection. Data obtained from this study were analyzed using the statistical package for social sciences (SPSS, version 20). Descriptive statistics were presented as mean ± standard deviation, frequency and percentage. P-value $\leq 0.05$ was considered statistically significant.

## Results

Our study included 111 patients who underwent TT procedure. 77.5% of the patients were male and 22.5% were female. Among the 50 study participants, 25 (50%) were male while 25 (50%) were female, with age frequencies ranging between (18-25 years), mean (21.34±1.61 years). Results revealed that RBC count showed no significant differences after 6, 12, and 24 hours compared to the baseline (P = 0.203, 0.244, 0.943; Table 1). Similarly, hemoglobin concentration remained unchanged across storage times compared to baseline (P = 0.812, 0.119, 0.511; Table 2). In contrast, PCV showed highly significant differences after 12 and 24 hours of storage (P < 0.001; Table 3). MCV also demonstrated significant changes at all storage times (P = 0.002, < 0.001, 0.001; Table 4). MCH values were not significantly affected across storage times (P = 0.331, 0.824, 0.106; Table 5), while MCHC showed highly significant changes after 12 and 24 hours (P < 0.001, 0.002; Table 6). Likewise, RDW-CV revealed highly significant variations after 12 and 24 hours (P < 0.001, 0.001; Table 7).

**Table 1. RBCs count between baseline and different storage times**

| | RBCs Count | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 6.398 | 8.8097 | 0.203 |
| | After 6 hours | 50 | 4.795 | 0.5941 | |
| **2** | Immediately | 50 | 6.398 | 8.8097 | 0.244 |
| | After 12 hours | 50 | 4.927 | 0.8313 | |
| **3** | Immediately | 50 | 6.398 | 8.8097 | 0.943 |
| | After 24 hours | 50 | 6.555 | 12.6366 | |

**Table 2. Hb values between baseline and different storage times**

| | Hemoglobin concentration | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 14.166 | 1.8917 | 0.812 |
| | After 6 hours | 50 | 14.176 | 1.9740 | |
| **2** | Immediately | 50 | 14.166 | 1.8917 | 0.119 |
| | After 12 hours | 50 | 14.226 | 1.9739 | |
| **3** | Immediately | 50 | 14.166 | 1.8917 | 0.511 |
| | After 24 hours | 50 | 14.206 | 1.9938 | |

* P value ≤ 0.05

**Table 3. PCV values between baseline and different storage times**

| | Packed cell volume | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 40.532 | 4.6265 | 0.169 |
| | After 6 hours | 50 | 40.780 | 4.8267 | |
| **2** | Immediately | 50 | 40.532 | 4.6265 | < 0.001 |
| | After 12 hours | 50 | 41.238 | 5.0757 | |
| **3** | Immediately | 50 | 40.532 | 4.6265 | < 0.001 |
| | After 24 hours | 50 | 42.424 | 5.9072 | |

* P value ≤ 0.05

**Table 4. MCV values between baseline and different storage times**

| | Mean red cell volume | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 84.250 | 5.5873 | 0.002 |
| | after 6 hours | 50 | 84.536 | 5.7768 | |
| **2** | Immediately | 50 | 84.250 | 5.5873 | < 0.001 |
| | after 12 hours | 50 | 85.240 | 5.9687 | |
| **3** | Immediately | 50 | 84.250 | 5.5873 | 0.001 |
| | After 24 hours | 50 | 88.056 | 9.1032 | |

* P value ≤ 0.05

**Table 5. MCH values between baseline and different storage times**

| | Mean cell haemoglobin | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 29.362 | 2.7231 | 0.331 |
| | After 6 hours | 50 | 29.568 | 2.5928 | |
| **2** | Immediately | 50 | 29.362 | 2.7231 | 0.824 |
| | After 12 hours | 50 | 29.376 | 2.7258 | |
| **3** | Immediately | 50 | 29.362 | 2.7231 | 0.106 |
| | After 24 hours | 50 | 29.932 | 3.6863 | |

* P value ≤ 0.05

**Table 6. MCHC values between baseline and different storage times**

| | Mean cell haemoglobin concentration | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 34.806 | 1.4223 | 0.346 |
| | After 6 hours | 50 | 34.718 | 1.2759 | |
| **2** | Immediately | 50 | 34.806 | 1.4223 | < 0.001 |
| | After 12 hours | 50 | 34.432 | 1.2384 | |
| **3** | Immediately | 50 | 34.806 | 1.4223 | 0.002 |
| | After 24 hours | 50 | 33.600 | 2.9888 | |

* P value ≤ 0.05

**Table 7. RDW-CV values between baseline and different storage times**

| | Red cell distribution width- CV | N | Mean | Std. Deviation | P value * |
|---|---|---|---|---|---|
| **1** | Immediately | 50 | 13.230 | 0.9624 | 0.187 |
| | After 6 hours | 50 | 13.702 | 2.6974 | |
| **2** | Immediately | 50 | 13.230 | 0.9624 | < 0.001 |
| | After 12 hours | 50 | 13.738 | 1.0945 | |
| **3** | Immediately | 50 | 13.230 | 0.9624 | < 0.001 |
| | After 24 hours | 50 | 14.112 | 1.0107 | |

## Discussion

Complete blood count (CBC) testing represents one of the most routinely performed laboratory investigations, providing fundamental and valuable information that not only facilitates diagnosis and guides further testing but also plays an essential role in patient monitoring (Lippi et al., 2006). Given that blood tests are more frequently conducted than analyses of other biological fluids, it becomes essential to establish the optimal storage conditions, including temperature and duration, to preserve sample integrity (Mosca et al., 2009). For the most reliable hematological results, whole blood specimens should ideally be analyzed immediately after collection, as significant delays or improper storage can result in imprecise, inaccurate, and ultimately unreliable data, potentially compromising clinical decisions (International Council for Standardization in Haematology et al., 2014). Consequently, understanding which hematological parameters are prone to clinically significant alterations during storage at ambient temperatures, and which remain relatively stable under the same conditions, is of critical importance. The present study aims to investigate the changes occurring in red blood cell parameter (RBC count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width-CV) and to evaluate the validity of results obtained from samples stored at room temperature that could not be analyzed immediately, in comparison to baseline measurements.

The findings of this study demonstrated that at different storage times (6, 12, and 24 hours) at room temperature (24°C), several hematological parameters showed significant changes. RBC count remained stable throughout the 24-hour study period compared with the immediate measurement (P = 0.943), with a mean of 6.40 ± 8.81 immediately versus 6.56 ± 12.64 after 24 hours, this result is in agreement with previous studies made by Baca (Baca et al., 2006) reported that RBC count remained relatively stable throughout their study period. Similarly, Joshi (Joshi et al., 2015) observed that red cell count was stable for up to 72 hours and Gunawardena (Gunawardena et al., 2017) reported stability of RBCs at room temperature for up to 48 hours. Hemoglobin concentration also remained stable over the 24-hour period with a mean of 14.17 ± 1.89 immediately versus 14.21 ± 1.99 after 24 hours (P = 0.51). This stability can be explained by the fact that hemoglobin is a protein and its concentration does not change significantly over time. This finding is consistent with several previous studies (Mahmoodi et al., 2006; Lippi et al., 2006; Imeri et al., 2008; Schapkaitz & Pillay, 2015; Pintér

et al., 2015). For packed cell volume (PCV), a highly significant difference was observed between the immediate measurement and the value after 12 hours ($P < 0.001$), with a mean of 40.53 ± 4.63 immediately versus 41.24 ± 5.07 after 12 hours. The significant increase in PCV after 12 hours is likely a consequence of changes in MCV, since PCV is derived from MCV. This finding is consistent with previous study (Baca et al., 2006) who reported that PCV exhibited clinically significant changes beginning on day 1 (24 hours) after blood collection, and agree with Pintér (Pintér et al., 2015) who observed a significant increase in PCV after 8 hours of storage at room temperature. The study also demonstrated a significant increase in MCV values after 6 hours compared to the immediate measurement ($P = 0.002$), with a mean of 84.25 ± 5.59 immediately versus 84.54 ± 5.78 after 6 hours. This increase in MCV likely reflects red blood cell swelling at room temperature. The finding is consistent with Baca (Baca et al., 2006) who reported significant changes in MCV within the first day, and with Imeri (Imeri et al., 2008) who noted that MCV stability at room temperature is guarantee only for a few hours. Conversely, this result disagrees with Schapkaitz and Pillay (Schapkaitz & Pillay, 2015) who observed no change in MCV during the first 12 hours of storage regardless of temperature, which may be attributed to differences in the use of an electronic hematology analyzer and sodium heparin as an anticoagulant. In terms of MCH, values showed no significant changes across different storage times and remained stable throughout the 24-hour study period compared with the immediate measurement ($P = 0.106$), with a mean of 29.36 ± 2.72 immediately versus 29.93 ± 3.69 after 24 hours. The stability of MCH can be explained by the stability of hemoglobin concentration and RBC count in samples stored at room temperature. This result agrees with Baca (Baca et al., 2006) who reported MCH stability for up to 4 days, and is consistent with previous studies (Mahmoodi et al., 2006; Schapkaitz & Pillay, 2015) who noted that MCH is minimally affected by storage temperature and time, allowing analysis up to 48 hours after collection at room temperature. Conversely, this finding disagrees with recent study (Schapkaitz & Pillay, 2015) who observed a significant increase in MCH after 24 hours of storage.

Prolonged storage, particularly at high ambient temperatures, may lead to hemolysis and a subsequent decrease in RBC count, which could explain the increase in calculated MCH (Bain et al., 2011). Additionally, this finding is inconsistent with Pintér (Pintér et al., 2015) who reported a significant decrease in MCH when stored at room temperature. Regarding MCHC, the study demonstrated a highly significant difference between the immediate measurement and the value after 12 hours ($P < 0.001$), with a mean of 34.81 ± 1.42 immediately versus 34.43 ± 1.24 after 12 hours. The observed decrease in MCHC after 12 hours may reflect the concurrent increase in PCV. This finding is consistent with Pintér (Pintér et al., 2015) and Mahmoodi (Mahmoodi et al., 2006) who reported that MCHC remains stable up to 8 hours, and with Baca (Baca et al., 2006) who observed significant changes in MCHC beginning on day 1 after collection. Conversely, this result disagrees with previous study (Schapkaitz & Pillay, 2015) who reported a significant increase in MCHC after 24 hours of storage at 27°C. This discrepancy can be attributed to higher storage temperature and prolonged duration, which may lead to hemolysis, a decrease in RBC count and PCV, and a consequent increase in calculated MCHC (Bain et al., 2011). Finally, RDW-CV study showed a highly significant difference between the immediate measurement and the value after 12 hours ($P < 0.001$), with a mean of 13.230 ± 0.96 immediately versus 13.738 ± 1.09 after 12 hours. The increased MCV could be the possible cause of significant increase in RDW-CV values after 12 hours. These findings are consistent with those reported by Gunawardena (Gunawardena et al., 2017) who noted that RDW-CV were stable up to 6 hours and increased after 24 hours of storage. Similarly, agree with Baca (Baca et al., 2006) who observed a significant increase in RDW-CV over time beginning on day 1 after blood collection.

## Conclusion

The study concluded that there were no significant differences in RBC count, hemoglobin concentration and MCH in samples stored at room temperature throughout the 24-hour study period. PCV showed significant changes after 12 hours, indicating stability up to 6 hours. MCV exhibited significant differences after 6 hours, suggesting that its stability is guaranteed only for a few hours. Similarly, MCHC and RDW-CV showed significant changes after 12 hours, remaining stable up to 6 hours. Based on these findings, it is recommended to perform CBC analysis on blood specimens as soon as possible after collection. Further studies are recommended to evaluate the actual stability of the parameter when the storage period is reduced to less than 6 hours.

## Author contributions

Each author made a significant intellectual contribution, reviewed and approved the final manuscript version, and consented to take responsibility for all elements of the work.

## Funding

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## Conflict of interest

The authors declare no conflict of interest. The manuscript has not been submitted for publication in other journal.

## Ethical concern and ınformed consent

Ethical approved was obtained from the Research and Ethic Committee in Faculty of Medical Laboratory Sciences, University of Gezira. All participants provided written informed consent, and the study followed the Declaration of Helsinki ethical guidelines.

## AI tool usage declaration

The authors declare that no AI tools were used to generate scientific content, data interpretation, or reference citations in this manuscript. However, AI-assisted tools such as Grammarly, ChatGPT, and QuillBot were used solely for grammar correction, language refinement, and improving readability. All scientific content was written by the authors, who carefully reviewed and verified the manuscript for accuracy, originality, and completeness.

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