

## The Effect of Storage Temperatures and Time on Complete Blood Count Parameters

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### ABSTRACT

#### Aim

The aim of the study is to assess the stability of Complete Blood Count under the storage temperature and period of storage.

#### Methodology

The study was carried out at the haematology laboratory of Mubarak Alkabeer Hospital—a total of 18 samples of male patients' blood in the EDTA tubes. There were two groups of samples: The refrigerated group, which stored the sample at 4-8°C, and the room temperature group, in which a sample was kept at room temperature, 21°C. The parameters of the Complete Blood Count included White Blood Cells, Red Blood Cells, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Red Cell Distribution Width, Platelet Count, and Mean Platelet Volume were measured daily for five days. For the refrigerated group, stability at time points of more than five days was considered. The analysis was performed using a Unicell DxH800 analyzer.

#### Results

The stability of a Complete Blood Count can be dependent on how it is stored. Both White Blood Cells and Mean Platelet Volume. Mean Platelet Volume had a significant variability, with White Blood Cell count being reduced by Day 4 and Mean Platelet Volume increased by Day 5. Red Blood Cells and Hemoglobin maintained relatively constant levels in both conditions. Still, there were more pronounced differences in Mean Corpuscular Volume and Mean Corpuscular Hemoglobin, especially when left at room temperature. Hematocrit was found to fluctuate under environmental conditions, while Mean Corpuscular Hemoglobin declined consistently. A relative difference of a Red Blood cell parameter was observed in the form of a Red Cell Distribution Width elevation, suggesting that variation in the size of the red cells has occurred over time. The samples refrigerated at 4-8°C showed higher stability for almost all the parameters of Complete Blood Count than at room temperature

#### Conclusion

It is preferable that the Complete Blood Count testing be performed on the day of collection. Parameters including White Blood Cells, Mean Corpuscular Volume and Mean Platelet Volume gravity declined significantly at the ambient temperature, raising the imperative required to make sure to adhere correctly to the storage procedures for proper test value results.

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the viability of the cell may vanish, affecting the authenticity of the test result [4].

### Introduction

The most frequently employed laboratory test to assess the well-being of an individual is the Complete Blood Count (CBC). The CBC is valid for the diagnosis of various health issues, including anaemia, leukaemia and different blood disorders [1]. The amount of sample stored has also been observed to influence the results of the test. Various conditions may considerably affect the outcome of the test, which includes variation in the size of the sample, testing principles, usage of analyzers and delays in shifting the blood sample from the phlebotomy room to the central lab or loss of sample in the lab [2]. Even slight delays may considerably affect the accuracy of CBC results since certain elements are sensitive to timings. The haematological elements are dependent on the duration of collection and analysis as well as the storage conditions [3]. A storage temperature of blood is 4-8°C for 24 hours, above which

The critical factor that affects the results of CBC is temperature control. Errors usually occur if storage units are not adequately managed, power failures arise, or the technicians are setting improper temperatures mistakenly [5]. When samples are being tested on the wrong days and duration, it can lead to incorrect results, highlighting the importance of accurate duration, transportation and temperature protocols for the laboratory samples. A structured framework and proper sample handling are crucial aspects to enhance the reliability of the test results [6]. A proper protocol with a specific time is followed for the processing of the blood sample to obtain accurate results [7]. The blood specimens are usually stored in a refrigerator or at room temperature. Incidental exposure to extreme temperatures may cause destruction of the cellular components present in the blood [8]. The alterations can affect the parameter values in the CBC, therefore leads to inaccuracy of the results [9].

Past study shows that the parameters of the blood can be preserved effectively in the refrigerator than in the environmental condition [10]. Moreover, the influence of prolonged storage under both the conditions on certain components of the blood including platelets and WBCs is not completely understood [11]. The causative factors which lead to the degradation process during the blood storage is intricate. Following factors such as enzymatic activity, oxidative stress and the mechanical factors of the storage container cause the degradation of the components present in the cell [12]. Platelets are sensitive in nature when exposed to varying degrees of temperature [13]. The WBCs and RBCs me change morphologically and loss of viability when exposed to improper condition of the storage [14].

The alterations in the cellular componets may lead to inaccurate testing outcomes [15]. There is a considerable study gap in understanding the effect the both time and temperature on specific CBC parameters [16]. Moreover, past literature has emphasized on the short-term storage and limited data is present on the freezing, refrigeration and environmental storage [17].

The present study analyses the CBC parameters to assess the method for accepting or rejecting the specimen under various storage conditions, emphasizing the duration and temperature. The conditions systemically vary across multiple days and degrees, therefore recognizing which elements are reliable and which are unreliable for analysis. The study is novel as there is a lack of sufficient data on the topic in Kuwait. The outcome of the study helps in refining existing guidelines regarding existence and rejection in the laboratories. The study also offers updates about advanced technologies, which improves the stability and authenticity of the test.

### Methodology

The study was executed at the haematology laboratory of Mubarak Alkabeer Hospital. The study was designed to determine the stability of parameters when blood samples were subjected to various conditions over a specific time.

Out of the total 48 residual blood samples, 18 samples were chosen for the analysis. These samples were collected from male patients of different age groups as part of the usual caseload of the hospital's haematology laboratory. The samples were collected on EDTA tubes in order to stop clotting and maintain the samples as stable as possible during storage and analysis. Both standard and abnormal cases were included to enrol various levels of CBC parameters and to offer an extensive review of the precision of the different CBC parameters across typical imprecisions.

All the tests reported in this study were carried out on the Unicell DxH800 analyzer, which is fully automated and is one of the most accurate haematology analyzers available. This analyzer is preferred because of its accuracy and speed when it comes to complete blood counts and the ability to help calculate important haematology indexes.

The 18 collected blood samples were divided into two primary groups based on the storage temperature:

#### Refrigerated Storage Group

As for general laboratory refrigeration conditions, samples were kept at the temperature of 4-8°C.

#### Room Temperature Storage Group

This was accomplished by keeping samples at an average temperature of 21°C that is close to the ambient room temperatures usually found in the laboratory.

### Analysis Schedule

Daily testing on each group of samples was performed over five days to determine the stability of CBC parameters. Because of this factor, the tests were conducted at a similar time in the day in order to eliminate any effects that may be related to timing. This made it possible to compare CBC values directly day by day in order to assess changes throughout the study.

### Long-Term Storage Analysis

Besides the day's stability assessment, some samples in the refrigerated storage group were reserved for the long-term experiment. Some of these samples were allowed to decompose for two months. They were then retried to establish the impact of the procedure on the variability of CBC parameters. One of the objectives of this part of the study was to investigate whether blood samples could be tested outside the short-term range and what changes may be caused by storage.

### Parameters Assessed

The following parameters were chosen based on their relevance in clinical diagnostic services and likely vulnerability to degradation from storage conditions:

Haemoglobin (Hb), Red Blood Cells (RBC), White Blood Cells (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), Red Cell Distribution Width (RDW) and Mean Platelet Volume (MPV).

To control for variability, each sample was analyzed using the same procedure. The Unicell DxH800 analyzer subsequently analyzed each of the blood samples, and the data was collected on a daily basis. As for each of the tests, the obtained results were compared with the values assessed on the first day, and the possible variations or trends in the stability of the parameters were revealed.

Therefore, measures to enhance the quality of the study were exercised throughout the study. Analytical measurements performed on the Unicell DxH800 analyzer were frequently checked and adjusted to ensure high precision. Samples were processed according to procedures that would minimize the pre-analytical variation. For instance, sample handling duration and transfer for storage, in addition to other kinds of sample transfers, were standard. Temperatures inside the refrigerators and rooms of both the refrigerated and room temperature groups were checked regularly with calibrated thermometers in order to detect sources of variation. Also, those technicians who were conducting the tests only knew the day that each sample was stored but were not told the specific day it was taken.

### Results

Table 1 illustrates the changes in different blood parameters upon storage in a refrigerator for five days. The indicators were WBC, RBC, haemoglobin, packed-cell volume or hematocrit, MCV, MCH, MCHC, RDW, platelet count, and MPV. Biasmax% is the column with the maximum allowable bias or deviation from the original value of the parameter. Daily change is documented from Day 2 through to Day 5, and the data provides averages and ranges of the observed change.

Regarding WBCs, a negative trend between Days 2 and 4 was observed, which indicated a decline in the WBC count within the first four days. It was observed that on Day 2, the score was -4.27, and on Day 4, the value decreased to -4.44; therefore, on the fifth day, it enhanced slightly to a positive value of 0.386. The ranges show increasing fluctuation with time from -

17.8 by Day 4 and a high of -6.666 by Day 5. This implies that WBCs may decrease or increase the rate of variation in the refrigerator, and this can distort the blood analyzing results when the samples are kept for too long.

RBCs vary only slightly across the observed days, with the daily averages fluctuating near zero values. For example, the change for Day 2 is equal to 0, and for Day 3, the change is - 0.185. From day 1 to day 5, there was a slight increase in positive opinion, 74.2%. The following variation, -4.1 to -1.298 on Day 2 and -2.929 to 2.6978 on Day 5, indicated that RBCs were more or less constant under conditions of cold temperature fluctuations. H.B. also recorded a slight negative trend in the observations made during the study. Day 2 and Day 5 both are equal to 0, and intermediate days show small negative values such as -0.632 on Day 3 and Day 4. The range of variation for haemoglobin, which ranges between -2.5 and -2.1 on Day 2, for instance, means that this parameter does not vary a lot in five days of storage. This stability may show that it is possible to use these measurements when samples are stored for this period.

Concentration enhancements are evident, with hematocrit levels

rising from 0,85 on Day 2 to 2,912 on Day 5 and range expansion, indicating growth in packed cell volume. The MCV also increases to 3.321 from 1.499, meaning that as storage increases, the RBC volume also increases. The MCH remains constant and increases only slightly from 0.346 on day 2 to 0.37 on day 5; the MCHC moderately reduces and fluctuates between -1.17 to -2.5423 with significant variability at different time intervals.

The results show that while RDW worsened from 1.43 to 2.158, effective indicated a more significant variance in the size of the RBCs. The platelet count reduces from - 4.05 on day 2 to - 2.4767 on day 5; hence, it tends to decrease over the days. MPV is negative in value from baseline with a value of -2.35 on Day 2 but becomes positive and reaches 14.457 on Day 5, also indicating variability by the end.

These results demonstrate that although some blood parameters like RBCs and H.B. remain relatively unchanged during storage, others like MPV and WBC counts fluctuate significantly. It is essential to draw accurate blood tests from the stored samples to understand these trends.

**Table 1: Differences of Parameters Stored in the Fridge**

Parameter	Biasmax%	Day 2	Day 3	Day 4	Day 5
White Blood Cells	6	-4.27	-2.22	-4.44	0.386
		-11.5 -- 1.35	-7.35 -- 3.3898	-12.32 -- 0	-17.8 -- - 6.666
Red Blood Cells	1.7	0	-0.185	-0.2832	0.742
		-4.1 -- 1.298	-4.1 -- 0.77	-2.929 -- 2.6978	-2.929 -- 2.6978
Haemoglobin	1.8	0	-0.632	-0.6329	0
		-2.5 -- 2.1	-2.88 -- 3.896	-2.05 -- 2.127	-0.63 -- - 5.597
Hematocrit	1.7	0.85	1.405	3.24	2.912
		-2.92 -- 4.51	-2.335 -- 5.90	-1.46 -- 5.90	-0.626 -- 8.68
Mean Corpuscular Volume	1.2	1.499	1.384	2.214	3.321
		-0.4587 -- -5.02	-1.3189 -- -6.37	-0.11 -- 6.37	1.26 -- 7.72
Mean Corpuscular Hemoglobin	1.3	0.346	0.35	0.346	0.37
		-1.41 -- 3.15	-2.73 -- 4.119	-2.1 -- 2.25	-1.02 -- - 3.157
Mean Corpuscular Hemoglobin Concentration	0.4	-1.17	-1.1799	-2.395	-2.5423
		-2.147 -- 0.906	-6.886 -- 1.1869	-3.9877 -- 0.604	-6.13 -- - 0.906
Red Cell Distribution Width	1.7	1.43	1.5267	2.56	2.158
		-2.0979 -- -5.73	0 -- 8.67	0 -- 8.67	0.72 -- 11.8
Platelets	5.9	-4.05	-2.7863	-1.03	-2.4767
		-10.79 -- 6.8	-12.162 -- -4.8	-12.16 -- 2.8	-12.16992 -- -8.8
Mean Platelet Volume	2.2	-2.35	3.529	5.88	14.457
		-21.7 -- 8.4	-19.13 -- 1.25	-16.52 -- 18.072	-13.91 -- 22.89

The table presents the dependency of the haematological parameters stored at room temperature for several days; the per cent variations of the parameters WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelets, and MPV between Days 2 to 5 have been included. It can be seen that each parameter displays different stability and variability patterns regarding its response to the room temperature.

WBC fluctuation over Days 2 to 5 is observed, with a decline of 6.687 % on Day 2 and a slight improvement by Day 4 (-3.0 %), and worsened on the following day, Day 5 (-6.398 %). This trend defines substantial variability in WBCs that are stored at room temperature, probably due to cellular degradation processes. On the other hand, the cells related to RBC show minimal variation, which is why these count for a more minor fluctuation. The mean bias of RBCs is similar for days 2 and 3 at -1.66 and -1.72% maximum, respectively. The lower values compared to those of WBCs imply that RBCs are less susceptible to room temperature conditions because of their perhaps structural nature and resistance.

H.B. levels are shown to be consistent throughout the period between the second and the fourth days and only rise by 0.3759% on the fifth day. This might be due to the stability of the H.B. molecule over temperature in spite of cell organization, which at the cellular level is much more susceptible to changes with temperature fluctuations. However, variability appears by day 5 with the range from -2.05% to 3.5%, which suggests that there may be fluctuations once exposed to room temperature for an elongated period. Hematocrit values are significantly higher than the baseline; the highest value of 1.289 per cent recorded on the second day infused increases to the highest value of 9.965 per cent recorded on the fifth day infused. This may be due to factors like RBC swelling or those attributed to high room temperatures, which increase concentration and, thus, high hematocrit values. The limited range of variation extends these findings further and underlines the fact that there is an inherent variability and possible lower limits of hematocrit accuracy under these conditions.

Regarding the MCV ratio, it increases from 4.03 % on Day 2 to 11.257% on Day 5. This sort of growth may cause RBCs to experience swelling in cases where the devices are not in their refrigerated state, therefore increasing the cell volume. The expansion recalled possible variability in MCV measurements when exposed to room temperature for an extended period. I have chosen MCH, and it only increases slightly from 1.47 on day 2 to 1.89 on day 5. Again, the range is less than that of MCV or hematocrit, indicating that MCH variability is comparatively low and might be resistant to variability owing to the fact that MCH depends on both the concentration of haemoglobin and the RBC count, both of which are relatively stable.

On the other hand, the values of MCHC are decreasing progressively, ranging from -2.958% on day 2 to -8.875% on the fifth day, with a negative trend and highest variability. This pattern may be due to a swollen RBC, which reduces MCV more than haemoglobin concentration. RDW rises gradually from 9.558% on Day 2 to 19.5% by Day 5, suggesting that the size of RBCs has become less homogeneous, possibly due to morphological or cellular modifications by room temperature storage.

The platelets vary slightly; they fall to -3.125% on the second day and rise to 3.57% on the fourth day, followed by a negligible increase of 0.446 % on the fifth day. This variation may occur because of platelet clumping or degradation at room temperature surroundings. MPV reduces, suitable from -14.13% increase on Day 2 to -6.9% increase on Day 5, indicating either reduced platelet size or could be due to platelet degradation.

The values in the table suggest that individual haematological parameters are affected in varying manners due to storage at room temperature. WBC, MCHC and MPV show considerable decay, suggesting that these parameters are reasonably sensitive to storage conditions and can rapidly deteriorate if the blood samples are left uncovered and at ambient temperatures. RBC, haemoglobin, and hematocrit are more stable, but tiny variations suggest that exposing a sample to room temperature for an extended period harms the examinations.

**Table 2: Differences of Parameters Stored at Room Temperature**

Parameter	Biasmax%	Day 2	Day 3	Day 4	Day 5
White Blood Cells	6	-6.687	-3.157	-3.0	-6.398
		-14.55 -- 2.12	-10.798 -- -4.44	-8.3 -- 7.445	-10.798 -- 0.169
Red Blood Cells	1.7	-1.60	-1.72	-1.466	-1.169
		-3.16 -- 0.6688	-3.35 -- 0.936	-3.63 -- 1.003	-2.3 -- - 0.57
Haemoglobin	1.8	0	0	0	0.3759
		-2.05 -- 5.43	-2.127 -- 3.529	-1.50 -- 3.260	-2.05 -- 3.5
Hematocrit	1.7	1.289	6.053	7.7481	9.965
		0.6465 -- 6.597	1.66 -- 10.894	4.65 -- 13.6	3.9867 -- 29.166
Mean Corpuscular Volume	1.2	4.03	7.14	9.385	11.257
		0.5958 -- 9.8	3.1777 -- 14.637	3.67 -- 17.039	3.57 -- 16.04
Mean Corpuscular Hemoglobin	1.3	1.47	2.777	2.05	1.89
		0.38 -- 4.87	-1.14 -- 4.59	0.70 -- 3.47	0.70 -- 3.47
Mean Corpuscular Hemoglobin Concentration	0.4	-2.958	-4.73	-7.017	-8.875

		-6.86 -- 3.92	-9.55 -- 0.98	-12.5 -- 1.63	-13.128 -- -1.6
Red Cell Distribution Width	1.7	9.558	15.44	19.0	19.5
		4.1 -- 14.28	10.27 -- 18.79	10.27 -- 21.55	11.85 -- 25.37
Platelets	5.9	-3.125	0	3.57	0.446
		-9.019 -- 5.744	-21.7 -- 5.74	-23.5 -- 11.78	-26.5 -- 13.79
Mean Platelet Volume	2.2	-14.13	-7.21	-4.04	-6.9
		-17.699 -- -9.859	-15.789 -- 15.49	-10.6 -- 22.535	-12.89 -- 25.35

## Discussion

The results obtained in this study give a significant amount of insight into the criteria of rejection and acceptance as well as a more detailed understanding of the nature of all the indices being measured and the degree to which they can fluctuate with time. When deciding the significance of the results, the p-value indicates the statistical significance of the results. The comparison of the results with the bias max suggests the clinical relevance of the results. The main objective was to answer how many days the indices were accurate and what time or temperature affected their viability. After reviewing the results, it is clearly prominent that overall, the blood samples that were stored at 4°C in the fridge were more stable over a more extended period. At the same time, the samples stored at room temperature didn't show much stability.

When compared to the bias max, the WBC, RBC, HBG, MCH, and PLT indices stored in the fridge showed complete stability and reliability throughout the five days of testing. HCT and RDW were reliable and stable up to the third day of testing and have proved to become unreliable starting the fourth day of testing. As for the MCV and MCHC, they show unacceptable bias and should only be tested on the day of collection. After six h, meaningful bias was observed for MCH and mean corpuscular volume MCV [18]. As previously mentioned, another study also confirmed the unacceptability of the MCH and MCV.

As for the parameters at room temperature, which maintained a temperature of 21°C, HBG and PLT showed complete stability, making them reliable for up to five days. As for the RBC and HCT, they were found to be stable only on the second day. Finally, the WBC, MCV, MCH, MCHC, and MPV showed an unacceptable bias because they were only reliable on the day of collection.

Overall, HBG and PLT showed complete stability for the longest time despite the different temperatures to which the samples were exposed. As for the other parameters, they show varying reliability because they were stable for a different number of days between the end of the collection and the fifth day of testing. MCV, MCHC, and MPV indices showed no stability or reliability for either temperature. Research conducted at a hospital in Mumbai indicates that haemoglobin was the best-preserved parameter, followed by WBC and platelets [19].

The findings of the present study correlate with the results of past research, highlighting the novel results regarding the stability of CBC parameters at room and refrigerated temperatures. According to Chen et al. (2022), there is an association between CBC elements and temperature and duration of handling. The samples were observed to show better stability at a temperature range of 4-8°C [20].

The results of WBC in the current study align with the results of Mourya et al., which also illustrated that the WBC is sensitive and is prone to destruction when the temperature is not kept right. This indicates that proper temperature and handling are essential [21]. The samples that were refrigerated in the present study were observed to show less decrease in WBCs. It indicates that low temperature does weaken the destruction, but not wholly.

The RBCs demonstrated an increased level of tolerance with lesser alterations in bias. As per a study by Zhang et al., RBCs are greater stress resisters and have more excellent stability under different temperature conditions [22]. Moreover, the consistency of H.B. from the second to the fourth day was observed to be constant, with a very minor rise on the fifth day. The observation aligns with the study by Li et al., who suggested that H.B. was mildly impacted by the storage temperature, which might be due to their stable structural framework [23].

The stability of platelets contrasts with the study of Yu et al., who suggested that platelets are sensitive to the environment in which they are stored. However, certain platelet elements can resist longer storage times than the rest [24].

Hematocrit values were also found to be higher in this study. They showed variation with temperature, as well as possible artefacts such as cellular swelling or evaporation, which may affect concentration differences. These results share similar observations with Azzahrah et al., who also identified that hematocrit levels are sensitive to changes in room temperature because of osmotic effects on RBCs [25].

MCV and MCH demonstrated an increase over time and were found to be more affected at room temperature. In our study, we saw, on average, a significant rise in MCV from the baseline by day 5, which could be attributed to RBC swelling. This corresponds with the study by Kengwendey et al., who discussed fluctuations in MCV and MCH when blood samples are not cold. These shifts can then cause distortions in the RBC indices, especially when the samples are left outside the refrigeration for long periods [26].

This study examines the impact of storage duration and temperature on CBC; however, earlier research also reveals the influences of room temperature on MCHC and MPV. This is in line with Knychala et al., whereby MCHC stability decreases as the level of RBC swelling increases, as we observed the decline in MCHC by Day 5 [27]. Likewise, Kayadibi et al. and Naveed et al. also reported fluctuations in MPV with variations in storage conditions; however, the degradation we detected was much steeper than what previous works described as comparatively stable [28,29].

In a study by Arrigo et al., the changes in WBC and RBC degradation indicate how pre-analytical factors, including temperature, transportation and equipment, affect the stability of the parameters. Collectively, the results of this study underscore the importance of temperature management and time-sensitive handling; they also provide evidence for the need for cold chain adherence and demanding protocols concerning elevated WBC, MCHC or MPV, and endorse universal CBC precision regardless of environmental conditions [30].

### Conclusion

In conclusion, it is recommended that for complete result accuracy, blood samples should be tested for the complete blood count on the same day of blood withdrawal to ensure maximum stability. If storage was needed for further testing, 4°C has shown to be the optimum environment. As for testing specific indices, the particular days of reliability are listed above and may provide accurate and reliable results.

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