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Effect of Blood Sample Storage on Sample Stability for CBC and Blood Cell Morphology Analysis

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ABSTRACT
Hematological analysis is fundamental in clinical practice, but delays in sample processing can compromise accuracy. This study aimed to evaluate the impact of storage time and temperature on complete blood count (CBC) parameters and blood cell morphology. Blood samples from 250 healthy adults were collected in K2EDTA tubes and stored at room temperature (18-25°C) and refrigeration temperature (2-8°C). Peripheral blood smears were prepared and analyzed at 0, 8,16, 24, 48, 72 and 96 hours post-collection. Erythrocyte, leukocyte and platelet morphology were assessed using the Turgeon grading system and qualitative evaluation, respectively. Leukocyte differential counts were performed using an automated analyzer. Erythrocyte crenation was observed earlier and more frequently in samples stored at room temperature, starting at 8 hours, compared to refrigeration (starting at 24 hours). Loss of central pallor in erythrocytes occurred after 16 hours at room temperature and 48 hours under refrigeration. Leukocyte morphological changes, including cytoplasmic vacuolization, nuclear lobulation and pyknosis, were evident after 8 hours at room temperature and 24 hours under refrigeration. These changes impacted leukocyte differential counts, particularly at room temperature. Storage conditions significantly influence cellular morphology. Erythrocyte changes are attributed to membrane alterations, while leukocyte changes are linked to oxygen deprivation and metabolic stress. EDTA, the anticoagulant, also contributes to cellular damage over time. Immediate analysis of peripheral blood smears is recommended for optimal accuracy. If delays are unavoidable, refrigeration at 2-8°C for up to 24 hours is preferable to room temperature storage. This study emphasizes the importance of standardized pre-analytical procedures for reliable hematological analysis.

INTRODUCTION

Hematological reporting plays a crucial role in accurately diagnosing various diseases, including organic, parasitic and metabolic disorders. It involves analyzing complete blood counts (CBC) and peripheral smears, providing vital information for physicians and surgeons to diagnose, monitor and manage patients effectively. CBC is particularly essential for determining the quantity of blood needed for transfusion[1]. Sample stability, defined as the ability of a sample to retain its initial measured value within specific limits over a defined period under defined conditions, is critical for ensuring the accuracy and reliability of results, especially in cases of delayed evaluation[2]. Delayed sample analysis is not uncommon in clinical practice due to organizational or technical reasons, which can result in imprecise, inaccurate and unreliable results, ultimately affecting clinical decisions[3]. While EDTA is commonly used as an anticoagulant for automated cell counts due to its availability, ease of preparation, widespread use and afford ability, blood samples anti coagulated with EDTA exhibit less stability in parameters and may undergo changes in cell morphology when stored at room temperature for extended periods. This can lead to incorrect data interpretation, particularly affecting red blood cell morphology due to altered osmotic fragility[4]. Factors such as manpower shortages, equipment limitations and high environmental temperatures contribute to longer sample storage times[5]. Additionally, transportation delays to centralized laboratories may further prolong sample evaluation times, necessitating careful attention to pre analytical procedures. Refrigerating blood samples at has been suggested as a simple and cost-effective method to maintain hematological parameters and peripheral smear morphology[6]. Given the prevalence of blood tests in clinical practice, it is essential to determine suitable storage temperatures and durations. Hence, this study aims to evaluate the changes in blood cells morphology based on blood storage period and temperature.

MATERIALS AND METHODS

This study utilized samples from 250 consecutive individuals within normal laboratory ranges. Blood samples (3 ml each) were obtained from healthy adults using two tubes containing dipotassium ethylenediaminetetraacetic acid (K2EDTA) anti coagulant. Conducted at the Swami Ramanand Teerth Rural Government Medical College and Hospital, Ambajogai from July 2023 to December 2023, the study incorporated peripheral blood smear examinations from the outset of sample collection. Blood specimens in K2 EDTA-containing tubes were stored at room temperature (18-25°C) and refrigeration temperature (2-8°C). Subsequently, peripheral blood smear examinations were conducted at storage intervals of 8, 16, 24, 48, 72 and 96 hours. Peripheral blood smear preparations were stained with Field stain and examined under a microscope at 10x, 40x and 100x magnifications. Erythrocyte morphology was semi-quantitatively assessed using the Turgeon scale, while leukocyte and platelet morphology were qualitatively evaluated. Erba Manheim Elite 580 five part analyzer is used to determine the WBC, RBC and Platelet counts based on the Electrical impedance method and flow cytometry. Testing of leukocyte differential counts between room and refrigerator temperature storage was performed using paired t-tests and Kolmogorov-Smirnov tests ($p<0.05$). Ethical approval for this study is obtained from the ethical committee of Swami Ramanand Teerth Rural Government Medical College and Hospital, Ambajogai, and all participants provided written informed consent.

RESULTS AND DISCUSSIONS

The alterations in erythrocyte morphology were examined through peripheral blood smear analysis following the storage of complete blood count samples under varying temperature and duration conditions. The assessment of morphological changes was done based on Turgeon grading system. The morphology of normochromicnormocytic erythrocytes for blood specimens was examined after blood collection. After storing erythrocytes at room temperature for 8 hours, 247 (98.8%) samples exhibited erythrocyte crenation graded at +3, whereas refrigerated samples stored for the same duration showed the onset of erythrocyte crenation in 19 (7.6%) samples, graded at +2. The loss of central pallor in erythrocytes emerged as a morphological alteration observed in specimens subjected to prolonged storage. In specimens stored at room temperature, this phenomenon commenced after a storage period of 16 hours, with 77 (30.8%) samples showing a grading scale of +1, 106 (42.4%) samples showing a grading scale of +2, 34 (13.6%) samples showing a grading scale of +3 and 20 (8%) samples showing a grading scale of +4.

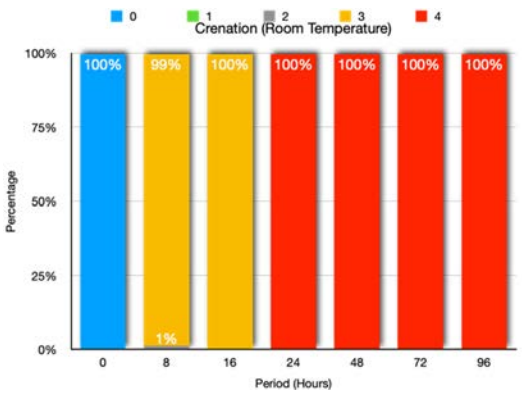

Fig. 1: Crenation (Room Temperature)

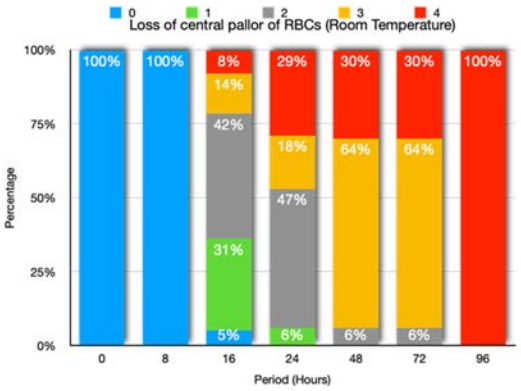

Fig. 2: Loss of Central Pallor of RBCs (Room Temperature)

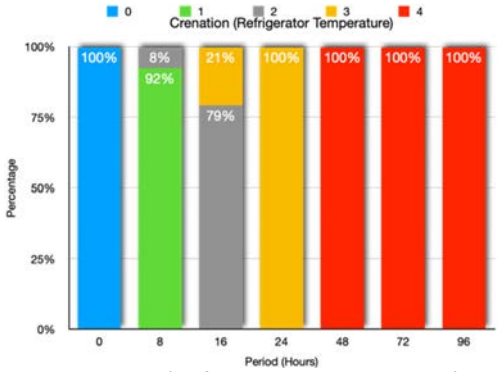

Fig. 3: Crenation (Refrigerator Temperature)

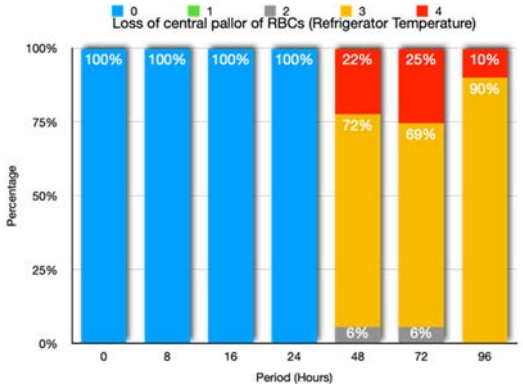

Fig. 4: Loss of Central Pallor of RBCs (Refrigerator Temperature)

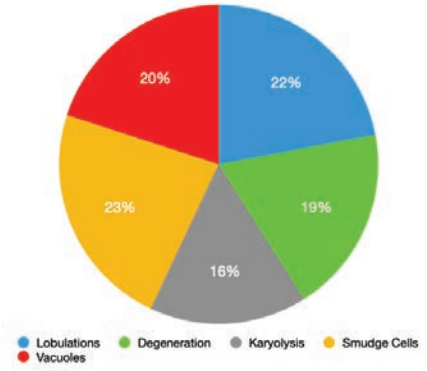

Fig. 5: Nuclear Changes in Leukocytes

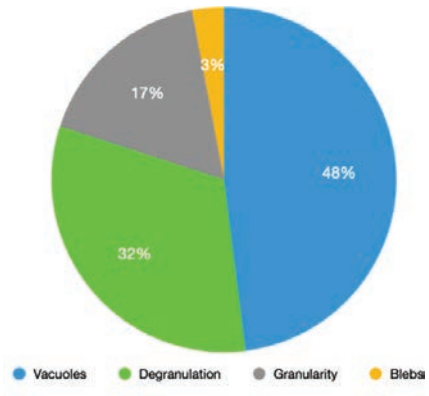

Fig. 6: Cytoplasmic Changes in Leukocytes

Conversely, in specimens stored at refrigerated temperatures, the loss of central pallor within erythrocytes became evident after a storage period of 48 hours, with 14 (5.6%) samples displaying a grading scale of +2, 180 (72%) samples exhibiting a grading scale of +3 and 56 (22.4%) samples showing a grading scale of +4. The morphological alterations in erythrocytes involved were evaluated utilizing the Turgeon scoring system, depicted graphically through bar charts, where scores ranged from 0 (blue) to +4 (Red), including intermediate values like +1 (green), +2 (gray) and +3 (yellow). The onset of morphological changes in leukocytes occurred after 48 hours of storage at temperatures between 2-8°C, whereas at temperatures between 18-24°C, changes began after 8 hours of storage. Some observed morphological alterations in neutrophils included core lobe separation, cytoplasmic boundary damage, disappearance of granules and visibility of small cytoplasmic vacuoles. Morphological changes in monocytes consisted of small vacuoles in the cytoplasm and irregular lobulated nuclei damage. Lymphocyte morphology exhibited slight alterations, such as cytoplasmic vacuoles, homogeneous nuclei and the emergence of 2-3 lobes in some nuclei. The changes observed in refrigerated samples were less pronounced, indicating better stability maintenance in refrigeration. The statistical significance was notably high (p=0.00002) in room temperature blood samples but reduced with refrigerated storage (p=0.0007), indicating sustained stability due to refrigeration. The results of the leukocyte differential count done on cell counter between room and refrigerator temperature showed that the statistical significance was unremarkable. The stability of erythrocyte morphology was found to be influenced by both storage temperature and duration. Our study revealed that alterations in erythrocyte shape began to manifest after eight hours of storage, with crenated erythrocytes forming under both ambient temperature and refrigerated conditions. Interestingly, crenation

Table 1: Storage Period and Temperature

		Neutrophils		Lymphocytes		Monocytes		Eosinophil		Basophil	
Storage Period	Temperature	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0 hour	2-8°C	57.4±7.469	0.3966	31.50±5.860	0.3272	5.74±1.450	0.2597	0.5	1	0.5	0.480
	18-24°C	56.8±8.285		32.00±5.539		5.90±1.695		0.5		1	
8 hours	2-8°C	54.33±8.656	0.4476	31.79±5.810	0.0574	6.23±1.426	0.0574	0.78±0.581	0.2597	1	0.5
	18-24°C	54.43±8.394		30.75±6.409		6.54±1.715		0.69±0.613		1	
16 hours	2-8°C	59.85±6.838	0.6203	32.89±5.982	0.5173	5.8±1.652	0.166	0.45±0.593	0.45	1	1
	18-24°C	59.54±7.137		32.55±5.741		5.75±1.459		0.44±0.620		1	
24 hours	2-8°C	57.3±7.268	0.5636	30.59±6.180	0.0047	6.01±1.826	0.439	0.39±0.675	0.39	1	0.439
	18-24°C	56.9±8.157		28.83±7.619		5.8±1.736		0.47±0.751		1	
48 hours	2-8°C	55.56±8.264	0.4967	31.12±7.237	0.2976	5.5±1.589	0.083	0.58±0.429	0.58	1	0.083
	18-24°C	56.05±7.824		30.47±6.672		5.7±1.680		0.64±0.561		1	
72 hours	2-8°C	58.8±6.648	0.5209	34.76±7.924	0.7301	6.2±1.924	1	0.55±0.630	0.55	1	1
	18-24°C	59.2±7.255		34.52±7.643		6.0±1.837		0.51±0.572		1	
96 hours	2-8°C	57.5±6.739	0.5027	31.70±6.510	0.7301	5.3±1.925	1	0.489±0.520	0.489	1	1
	18-24°C	57.9±6.548		31.56±6.420		5.4±2.140		0.56±0.490		1	

was more evident at ambient temperature compared to refrigeration. Additionally, after 16 hours of storage, loss of central pallor of erythrocytes was observed at ambient temperature, while they appeared after 24 hours at refrigerator temperature. This highlights the impact of storage conditions on cell aging and death, ultimately leading to morphological alterations. The morphological changes observed in erythrocytes were attributed to the loss of erythrocyte cell membrane lipids and increased membrane stiffness[7]. These changes initiated with an enlargement of cell size, eventually resulting in the formation of spherocytes and crenation. These alterations persisted even when specimens were refrigerated at 4°C, underscoring the importance of prompt peripheral blood smear analysis upon sample collection. EDTA causes irreparable harm to the biochemical, structural and functional aspects of other cells as well as blood and platelets. The erythrocytes were impacted by EDTA, resulting in crenation, spicules and echinocytes, also known as burr cells[8]. When samples containing EDTA were kept at room temperature for more than five hours, red blood cell artifacts were observed on peripheral blood smear examination. Storing blood samples with EDTA as an anticoagulant for 72 hours at 4-8°C would result in certain erythrocyte morphological alterations as well as unusual osmotic fragility[9]. On the first day, the peripheral blood smear test findings revealed normochromic normocytic erythrocytes. Leukocyte morphological alterations were observed after 24 hours of refrigerated storage at 2-8°C and after 8 hours at room temperature, with changes becoming more pronounced with longer storage times. These alterations led to discrepancies in leukocyte differential counts, particularly at room temperature, where cytoplasmic abnormalities and vacuolization hindered cell identification. Additionally, nuclear changes such as karyolysis and fragmentation contributed to misinterpretations in cell identification. Following blood collection, due to oxygen deprivation, which results in a drop in ATP (adenosine triphosphate) and a decrease in oxidative phosphorylation., there is a decrease in the Na+ pump, which would increase the entrance of Ca2+, H2O and Na+ as well as increase the efflux of K+ due to cell swelling[10]. Additionally, the reduction in ATP leads to anaerobic glycolysis, which in turn causes chromatin condensation, a drop in glycogen, a rise in lactic acid and a fall in pH[11]. Notably, neutrophil morphological changes were observed shortly after blood collection, with alterations becoming evident after 12-18 hours of storage. These changes were exacerbated by EDTA concentration and storage duration, emphasizing the importance of prompt analysis or refrigeration of blood samples to maintain cell morphology.

CONCLUSION

Immediate examination of peripheral blood smears following sample collection is essential for precise evaluation. Optimal smear quality can be achieved within 2-8 hours at room temperature and 12-24 hours under refrigeration post-collection. These findings emphasize the critical importance of careful sample storage practices in maintaining the integrity of blood cell morphology for accurate clinical assessments. Interpreting complete blood count and peripheral blood smear, especially with 24-hour samples stored at room temperature, is inadequate. Therefore, refrigeration of EDTA blood samples at 4°C is recommended whenever a foreseeable delay in sample interpretation arises, ensuring the provision of an accurate report.

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