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# STORAGE-INDUCED CHANGES IN HEMATOLOGIC PARAMETERS OF BLOOD

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**ABSTRACT**: Sometimes blood samples took more time for reaching to the laboratory so it was necessary to know that the time span of stability for whole blood count, differential count, reticulocyte and peripheral blood smear morphology for that storage period. Forty blood samples stored in EDTA were analyzed on an auto analyzer. The samples were stored at room temperature and at 4 °C – 8 °C. Samples was analyzed every 10 hours for 5 days. whole blood count parameters (red cell count, hemoglobin) and differential count parameters (percentages of basophils, lymphocytes and monocytes) were stable for at least 48 hours when stored at RT. Platelets were only stable for 12 hours and the white cell count was stable for 36 hours when stored at RT. Storing samples at 4 °C – 8 °C significantly increased the stability of most parameters, in particular, mean cell volume and percentage of reticulocytes. However, differential parameters were associated with lower stability at 4 °C – 8 °C. PBS morphology was compromised for both the storage conditions. This study concluded that blood samples stored in EDTA at 4 °C – 8 °C for five days are suitable for whole blood count but not as appropriate for differential count and morphology.

**KEYWORDS:** WBC, RBC, Storage time, Room Temperature

## INTRODUCTION:

Prolong-delay in analysis of hematological parameters of blood due to transportation from remote centers or due to storage. It causes changes in blood parameters and erroneous laboratory results1. The nature and extent of the changes vary with time and temperature of storage [2, 3]. To prevent such storage induced changes, blood is often stored at a low temperature and analyzed as early as possible after collection. Accurate measurement of whole blood count, differential count and reticulocyte parameters, as well as peripheral blood smear morphology, are essential for the correct interpretation of hematology results. It is recommended that whole blood count parameters such as red blood count , white blood count, hemoglobin and platelet count be analyzed 24 hours after sample collection when stored at room temperature (RT)[4,5].However, parameters useful for diagnosis and monitoring of hematological disorders, such as mean cell volume (MCV), reticulocyte and PBS morphology, are unreliable after 12 hours[6]. Osmotic swelling of red cells during storage at RT affects volume-dependent variables and results in misclassification of a microcytic anemia as normocytic and, similarly, a normocytic anemia as macrocytic[7]. Reticulocytes mature into red cells after 24 hours in circulation. The Clinical and Laboratory Standards Institute recommends that samples stored at RT should be analyzed for reticulocytes within 6 hours of collection[8] and morphologic analysis would be done within four hours, prior to the onset of EDTA-induced changes in red and white cell morphology8. Recent studies indicate that longer storage durations are acceptable when samples are stored at 4 °C – 8 °C [9, 10].

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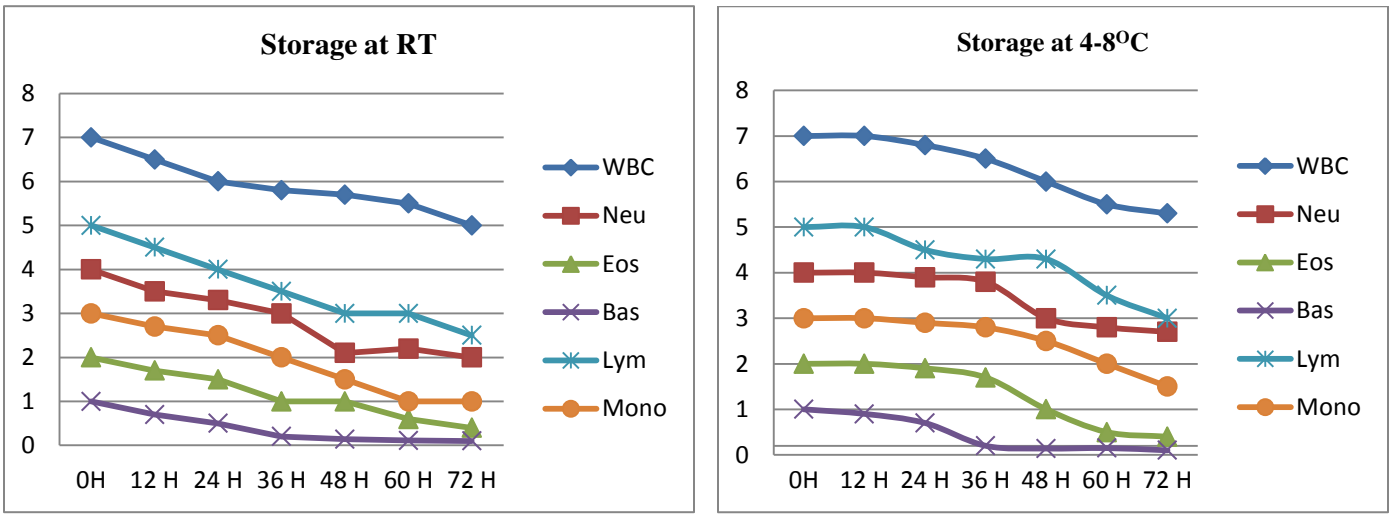
However, information on stability beyond 72 hours is limited. The aim of this study was to evaluate the stability of the whole blood count, differential count, reticulocyte and PBS morphology during extended storage at RT and at 4 °C – 8 °C in order to determine laboratory criteria for storage time and temperature for specimens referred for the work-up of hematological disorders from remote laboratories.

## MATERIAL AND METHODS:

This study was conducted at the Division of Haematopathology, Kasra General Hospital, Alvand St. Arjantin Sq. Tehran , Tehran , Iran in a routine diagnostic workups. The study associated with storage of blood at different temperatures and for different time-periods. 50 leftover blood samples of the patient population as well as normal population were taken. The samples collected in EDTA vials with adequate volume (> 4 mL) received within two hours of collection were included. Blood samples were evaluated for whole blood count, differential count and reticulocyte parameters. The parameters were analyzed with the laboratory's automated hematology analyzer [11,12]. The films were spread on the slide and stained by giemmsa staining for morphology. Samples were aliquot into two sets; one was stored at RT and other at 4 °C – 8 °C. Analyses of samples stored at RT and 4 °C – 8 °C were performed after 12, 24, 36, 48, 60 and 72 hours of storage. A manual differential count was performed on PBS of five samples stored at RT and at 4 °C – 8 °C. The PBS was first examined for the presence of EDTA-induced changes, including red cell spherocytes, echinocytes, sphero-echinocytes, increased rouleaux formation, degeneration of neutrophils and lobulation of lymphocyte nuclei,[10] because these changes preclude an accurate manual DIFF. Data were tabulated on Excel spreadsheets and analyzed using Statistical analysis were done using the software SPSS 20. The mean percentage difference from the value at time zero was calculated and tabulated. The changes in hematological parameters were analyzed with respect to the control (0-hour reading), in terms of storage time and storage temperature.

## RESULTS:

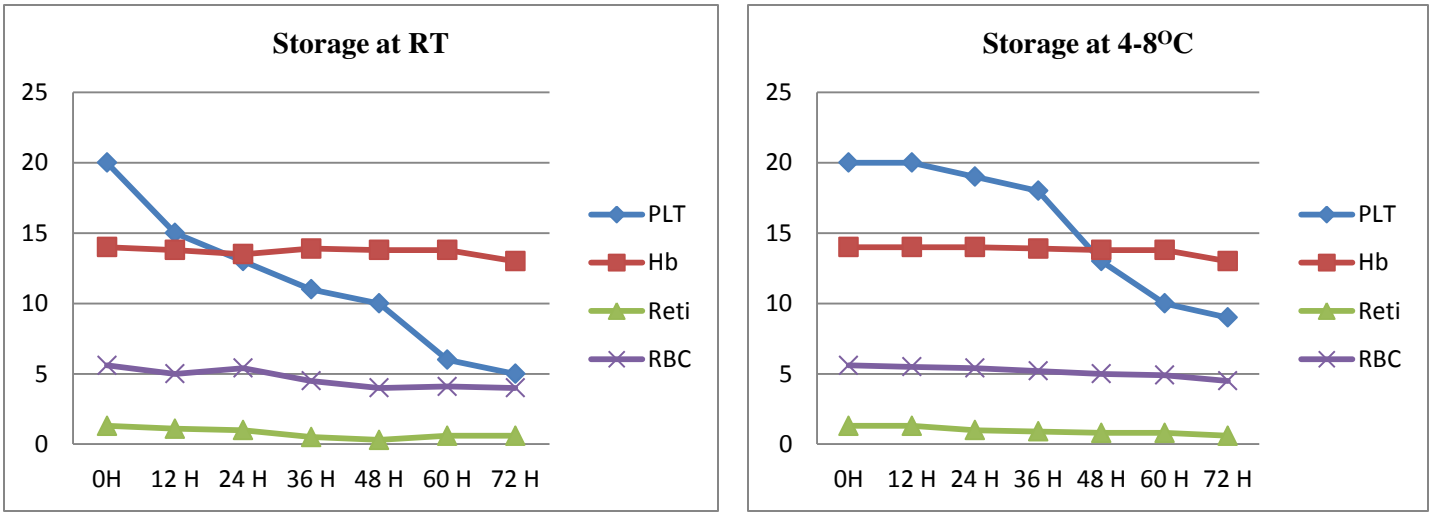
Most hematological parameters were stable up to 24 hours at 4 °C. There were insignificant changes in Hemoglobin concentration and RBC count up to 48 hours, in blood stored at 4 °C and 22 °C ($p > 0.05$). Reticulocyte count, Total WBC Count, Absolute neutrophil count and Platelet count varied significantly after 48 hours in all samples ($p < 0.001$). The common morphological changes in RBCs were spiculation or crenation and excessive rouleaux formation. WBCs showed nuclear degeneration (karyolysis and karyorrhexis) and cellular swelling. Platelets were swollen in some samples. MCV, MCHC and Mean cell hemoglobin (MCH) were stable for at least 48 hours after collection when stored at RT and were shows more stability when stored at 4-8°C. The hematocrit shows less effect by the storage temperature. The changes were more at higher temperatures and least when stored at 4-8 °C.




WBC White Blood Count 5-10*$10^3$/mm3 Differential WBC count:

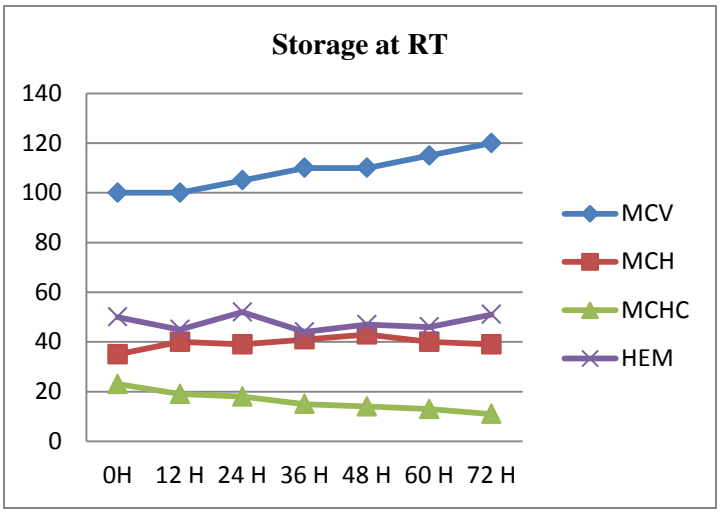
- Neu: Neutrophils 60–70% or 3,000–7,000/mm3( Active phagocytes; first to respond to inflammation or Infection)
- Eos: Eosinophil's 1–3% or 50–400/mm3 ( Respond to allergic reaction and parasitic infestations)
- Bas: Basophils 0.3–0.5% or 25–200/mm3 ( Respond to allergic and inflammatory reactions)
- Lym: Lymphocytes 20–30% or 1,000–4,000/mm3( Involved in immune reactions)
- Mono: Monocytes 3–8% or 100–600/mm3( Active in disposing of foreign and waste material)

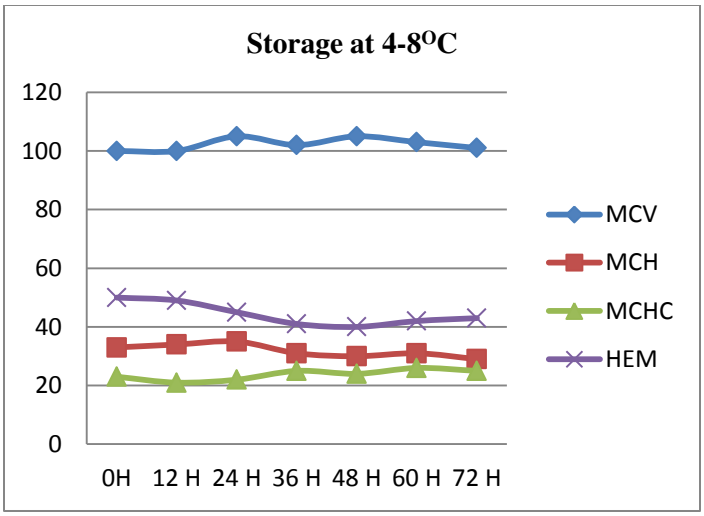
**Figure1. Changes in blood parameter at 4-8°C and room temperature**




- PLT: platelet count in lakh/cumm
- Reti:Reticulocyte count 1–1.5% of total (Number of immature RBCs in 1 mm3 of blood)
- Hb: Hemoglobin (Hgb) 14–16.5 g/dL(Amount of hemoglobin in 100 mL (1 dL) of blood)
- RBC: Red Blood Cell (RBC)4.2–5.4 million/mm3(Number of circulating RBCs per cubic millimeter of blood)

**Figure2. Changes in blood parameter at 4-8°C and room temperature**




■ MCV: Mean corpuscular volume 85–100 cubic micrometers Average volume of individual RBCs
■ MCH: Mean corpuscular 31–35 g/dL Weight of the hemoglobin in an average RBC
■ MCHC: Mean corpuscular hemoglobin 33.4–35.5% Average concentration (percent) of hemoglobin
■HEM: Hematocrit (Hct) Packed volume of RBCs in 100 mL of blood

**Figure3. Changes in blood parameter at 4-8°C and room temperature**

## DISCUSSION:

Due to population and less number of laboratories in many parts of our country necessitates collection of blood from remote centers and transport to referral laboratories. The delay in transport may cause time and temperature dependent alteration of the laboratory findings [1, 2, 3]. It is recommended that traditional FBC parameters be analyzed 24 hours after sample collection when stored at room temperature [1]. In our study we found that the platelet count, differential leukocyte count and reticulocyte count to change significantly in 24 hours' time. The reticulocyte count was most accurate within 6 hours of blood collection, and differential count within 12 hours. The parameters remained more stable at 4-8 °C. Platelets showed great variability in count and morphology. The changes were least, when platelets were examined within 12 hours and when stored at 4 °C. When the total count of WBC or platelets were too low or too high, an additional manual PBS examination under microscope increased accuracy of the test. The RBC morphology changes included crenation or spiculation and excessive rouleaux formation. These changes are also seen in different pathological conditions. Spiculated RBCs are often seen in uremia. Excess rouleaux formation may be a feature of chronic inflammatory disorders or multiple myeloma. Nuclear changes in WBCs included fragmentation, karyolysis and pyknosis. These changes often make differential count difficult and may lead to errors. Swollen platelets in a background of low platelet count often suggest a disorder of platelet formation, like Immune Thrombocytic Purpura. Thus, the morphological changes may confuse the pathologist and clinician to the exact nature of the disease. We can conclude that when blood samples are meant for routine

hematological tests, a peripheral smear should be prepared and stained within 4-6 hours. This can be used for differential count, approximate platelet count and morphological study of blood cells. If reticulocyte count has to be performed, it should be done along with the PBS. Rest of the parameters like Hb, RBC indices measured by auto-analyzers should be ideally measured within 24 hours of collection.. If there is any chance of delay, blood for all tests should be preserved at 4-8 °C.

**CONCLUSION:**

In conclusion, this study provides evidence regarding the viability of blood samples collected in EDTA vials and stored at RT and at 4 °C – 8 °C. Samples that have been stored at 4 °C – 8 °C for 72 hours are suitable for testing for whole blood count and reticulocyte parameters. However, this is not a solution for samples referred for PBS morphology review.

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