

A Prospective Assessment of the Effect of Room Temperature & Refrigerated Storage on Complete Blood Counts and on Morphological Features in Peripheral Blood Smear on Automated Haematological Parameters

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Abstract

Aim: The aim of the present study to assess the effect of room temperature & refrigerated storage on complete blood counts and on morphological features in peripheral blood smear on automated haematological parameters.

Methods: A prospective study was conducted at Department of Pathology, Darbhanga medical College and Hospital, Darbhanga, Bihar, India in between the duration of one year. Total 100 blood samples from outpatient & inpatient section irrespective of any specific criteria, age & gender were collected randomly.

Results: We observed that there was statistical significance (P values < 0.05) in WBC counts in blood samples stored at room temperature. Degranulation, increased granularity in neutrophils, vacuolization & bleb formation in cytoplasm & lobulation, pyknosis, smudging & vacuoles in nucleus are morphological changes seen WBC in stored blood samples at room temperature. The P values are not < 0.05 i.e no statistical significance in variation of haemoglobin values & RBC counts stored at room temperature for 24 hrs. However, some morphological artifacts in RBCs such as crenated RBCs & loss of central pallor revealed with stored sample at room temperature. While, P value < 0.05 i.e statistical significant changes seen in platelet counts in room temperature storage of blood & also shows morphological changes like aggregation of platelets & large platelets.

Conclusion: Blood samples stored at room temperature for 24 hrs results in changes in haematological parameters & morphology of cells. Hence, refrigerated storage at 4°C is recommended for accurate results in case evaluation of delayed samples.

Keywords: Sample stability, Storage conditions, Complete hemogram, EDTA anticoagulated blood.

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Introduction

Hematological reporting is very important aspect in accurate diagnosis & investigations of various organic disease, parasitic manifestations & metabolic disorders as well. [1] It includes complete blood count & peripheral smear which provides a definitive information to physicians or surgeons to diagnose, monitor & further management of patients. [2] Accurate hematological reporting is very important for management of patients. [3] Complete blood count and morphological assessment of peripheral blood smear examination are the most common investigations done in laboratory in order to give crucial information about disease and its final diagnosis. [4] Complete hemogram is also necessary for patients who requires blood

transfusion to estimate the quantity of blood to be transfused. [5] Proper sample collection and transporting it to the hematology department are very crucial to obtain high standard quality test results in the laboratory. [6] In routine hematological practice, EDTA is preferable choice of anticoagulant for automated cell counts due to its general availability, ease of preparation, wide spread use & comparatively low cost. [7,8] The stability of the parameters is restricted in samples of EDTA anticoagulated blood. [4,9] However, the EDTA anticoagulated blood shows less stability of parameters & shows changes in morphology of cells in stored blood sample at room temperature in case of delayed analysis & finally result in wrong interpretation of data. [2,8] Dipotassium EDTA

dihydrate is the anticoagulant of choice for modern automated CBC analyzers. [8] Hence, preanalytical phase needs a careful attention on procedures such as collection of blood samples, patient’s identification, preference of collection vials, labelling of specimens, accurate blood suction, clerical errors & storage. [1] Refrigerated storage of collected samples at 2-8°C maintains the stability of hematological parameters. Knowledge of the effects of temperature on blood sample stability is important in a hematology laboratory. A period of storage at high temperature may lead to variation in the different hematology parameters and may affect the accuracy of the final report. There is simple, modest method to maintain all hematological parameters & morphology of peripheral smear is storage of blood samples at 4°C refrigeration. [9]

Hence, this study aimed to assess the effect of room temperature & refrigerated storage on complete blood counts and on morphological features in peripheral blood smear on automated haematological parameters.

Material & Methods

A prospective study was conducted at Department of Pathology, Darbhanga medical College and Hospital, Darbhanga, Bihar, India in between the duration of one year. Total 100 blood samples from outpatient & inpatient section irrespective of any specific criteria, age & gender were collected randomly.

Methodology

Sampling: Approximately 2 ml of venous blood is collected in EDTA coated vacutainers under standard protocol of aseptic precautions.

Laboratory testing: The measurement of haematological parameters including WBC, RBC & Platelet count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) was done on Sysmex XN 500 5 part differential which is on based on the principle of fluorescence flow cytometry method. Furthermore, their peripheral smears are made by proper mixing of blood samples & using Leishman

stain. In peripheral smear following morphological changes were studied -

- RBC
 1. Crenated RBCs
 2. Loss of central pallor
 3. Spherocytic changes
- WBC
 1. Cytoplasmic changes such as vacuolization, degranulation, blebs.
 2. Nuclear changes such as lobulations, degeneration, karyolysis, vacuolization, smudge cells.
- Platelets
 1. Aggregation of platelets
 2. Large platelets

Room Temperature Storage

Analysis of 50 blood samples is done at 0 hr for complete blood count as basis of measurement by using automated haematological analyzer 5-part differential & peripheral smear is prepared. Then subsequently these samples were stored at room temperature & re-analyzed after 24 hrs for all haematological parameters & peripheral smear.

Refrigerated Storage

Analysis of another 50 blood samples is done at 0 hr for complete blood count as basis of measurement by using automated haematological analyzer 5-part differential & their peripheral smear is prepared. Subsequently samples were stored at 4°C refrigeration. After 24 hrs, samples were settled at room temperature for 30 mins, then reanalyzed for complete blood count & peripheral blood smear prepared.

Statistical Analysis

Statistical difference between values of the analyzed parameters at particular time intervals at 0 hr & at 24 hrs. SSPS software system was used to conduct paired T test to compare statistical results. If P value < 0.05, then it was considered as significant result.

Results

Table 1: Nuclear and cytoplasmic changes in WBC

Nuclear changes in WBC	%
Lobulations	17%
Degeneration	24%
Karyolysis	20%
Smudge cells	25%
Vacuoles	14%
Cytoplasmic changes	
Vacuoles	10%
Degranulation	40%
Granularity	16%
Blebs	32%

We observed that there was statistical significance (P values < 0.05) in WBC counts in blood samples stored at room temperature. Degranulation, increased granularity in neutrophils, vacuolization & bleb formation in cytoplasm & lobulation, pyknosis, smudging & vacuoles in nucleus are morphological changes seen WBC in stored blood samples at room temperature.

Table 2: Changes in RBC and platelets

Changes in RBC	%
Loss of central pallor in RBCs	42%
Crenated RBC	58%
Changes in platelets	
Aggregation of platelets	44%
Swelling of platelets	56%

The P values are not < 0.05 i.e no statistical significance in variation of haemoglobin values & RBC counts stored at room temperature for 24 hrs. However, some morphological artifacts in RBCs such as crenated RBCs & loss of central pallor revealed with stored sample at room temperature.

While, P value < 0.05 i.e statistical significant changes seen in platelet counts in room temperature storage of blood & also shows morphological changes like aggregation of platelets & large platelets.

Table 3: Result of samples stored at room temperature

Pair	Test	N	Mean value	P Values
Pair 1	RBC fresh	50	4.082510	0.0007
	RBC 24 hrs	50	4.255550	
Pair 2	Hb fresh	50	11.54342	0.0785
	Hb 24 hrs	50	11.34567	
Pair 3	HCT fresh	50	34.97367	0.0032
	HCT 24 hrs	50	35.91598	
Pair 4	MCV fresh	50	86.26390	0.0311
	MCV 24 hrs	50	86.34	
Pair 5	MCH fresh	50	28.48356	0.0044
	MCH 24 hrs	50	28.75366	
Pair 6	MCHC fresh	50	32.78	0.0240
	MCHC 24 hrs	50	33.81590	
Pair 7	RDW fresh	50	16.62367	0.0001
	RDW 24 hrs	50	17.50387	
Pair 8	WBC fresh	50	8396.620	0.1512
	WBC 24 hrs	50	8133.359	
Pair 9	PC fresh	50	210222	0.0001
	PC 24 hrs	50	219844	

Table 4: Result of samples stored at 2-4° C

Pair	Test	N	Mean value	P values
Pair 1	RBC fresh	50	4.28062	0.1020
	RBC 24 hrs	50	4.31676	
Pair 2	Hb fresh	50	11.47346	0.0875
	Hb 24 hrs	50	11.32105	
Pair 3	HCT fresh	50	36.17932	3.8964
	HCT 24 hrs	50	35.74816	
Pair 4	MCV fresh	50	86.09344	0.1354
	MCV 24 hrs	50	84.18490	
Pair 5	MCH fresh	50	28.11665	0.0970
	MCH 24 hrs	50	27.96665	
Pair 6	MCHC fresh	50	32.59	0.4724
	MCHC 24 hrs	50	32.048	
Pair 7	RDW fresh	50	16.37344	0.5970
	RDW 24 hrs	50	16.75355	
Pair 8	WBC fresh	50	9173.332	0.2136
	WBC 24 hrs	50	9041.820	
Pair 9	PC fresh	50	268733.3	0.1800
	PC 24 hrs	50	271356.8	

There was significant increase in MCV & decrease in MCHC, reduced WBC count & platelet count with storage at room temperature which was prohibited by refrigeration. However, both room temperature & refrigerated storage does not affect RBC count & hemoglobin.

Discussion

A complete blood count (CBC), also known as a full blood count (FBC), is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC. [10] Complete blood counts are done to monitor overall health, to screen for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments. [11] For patients who need blood transfusion, a blood count may be used to get data which would help plan an amount of treatment. In such cases, the person should have only one blood count for the day, and the transfusion of red blood cells or platelets should be planned based on that. Multiple blood draws and counts throughout the day are an excessive use of phlebotomy and can lead to unnecessary additional transfusions, and the extra unnecessary treatment would be outside of medical guidelines. [12]

We observed that there was statistical significance (P values < 0.05) in WBC counts in blood samples stored at room temperature. Degranulation, increased granularity in neutrophils, vacuolization & bleb formation in cytoplasm & lobulation, pyknosis, smudging & vacuoles in nucleus are morphological changes seen WBC in stored blood samples at room temperature. The P values are not < 0.05 i.e no statistical significance in variation of haemoglobin values & RBC counts stored at room temperature for 24 hrs. However, some morphological artifacts in RBCs such as crenated RBCs & loss of central pallor revealed with stored sample at room temperature. While, P value < 0.05 i.e statistical significant changes seen in platelet counts in room temperature storage of blood & also shows morphological changes like aggregation of platelets & large platelets. In recent years, a large central authorized laboratories encounters the common problems like proper transfer, storage duration of collected blood samples, long distance dispatchment of specimens which causes the delayed analysis of tests. [13] Therefore, delayed sampling is one of the common problems in routine clinical practice, if quick analysis is not possible or if sample is required for retesting. [14]

There was significant increase in MCV & decrease in MCHC, reduced WBC count & platelet count with storage at room temperature which was prohibited by refrigeration. However, both room temperature & refrigerated storage does not affect RBC count & hemoglobin. According to studies by Gulati et al and Turhan et al, parameters like RBC, WBC, Hemoglobin and platelets remain stable at room temperature even after 48 hours. [13,15] A study by Mane et al contrastingly state that statistically significant increased WBC and decreased RBC values were noticed at 27°C within 24 hours however there was no significant difference in hemoglobin concentration at 4°C and 27°C even up to 72 hours storage. [16] In another study by Joshi et al, among blood cell parameters tested, the white cell count, red cell count and hemoglobin levels were found to be stable for up to 72 hours however the mean cell volume (MCV) and hematocrit changed significantly following 24-h storage at room temperature. [17]

Our study has demonstrated that haemoglobin values & RBC counts are not affected in room temperature storage for 24 hours, but there is significant increase in MCV because of crenated RBC in blood samples stored at room temperature. This change is stabilized by storage of blood samples at 4°C refrigeration for 24 hours. These similar findings were observed in prior studies. [18] Other studies indicated that the degenerative changes in stored blood samples at room temperature allows the influx of water into cellular membrane result into increase in red cell size with time & eventually lead to increased mean corpuscular volume. & other morphological artifacts such as spherocytosis, echinocytosis & sphero-echinocytosis may found after 48 hr of storage at room temperature. [19]

Conclusion

The result of our study suggested that there are significant changes in haematological parameters from automated cell analyzer & their morphology of cells seen in peripheral blood smear, if blood samples stored at room temperature for extended period of 24 hrs after sample collection. Hence, storage of blood samples at room temperature should be avoided during transportation, within laboratory or in case of delayed evaluation. Finally, we have concluded that refrigerated storage at 4°C is the recommended method of preservation of blood samples for an accurate & standard test results in case evaluation of delayed samples.

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