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Pathology

Prolonged Storage- Induced Changes In Haematology Parameters And Stability At Room Temperature For Counting Red And White Blood Cells And Platelets

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ABSTRACT

A total of 100 blood samples from healthy volunteer (50 women and 50 men aged 18-60years) were obtained by vein puncture and samples collected in EDTA anticoagulated blood, specimens were processed through the sysmax cell counter at Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry. These subjects have given their informed consent. The blood samples were aliquot and were separated in to 2 parts, are kept at refrigerator temperature +4o C, other kept at +8o C and room temperature at +37° C. After collection of the blood samples an interval measurement was performed on sysmax cell counter hematology analyser. Subsequently, each sample was analysed for 6hrs, 12 hrs,48 hrs and 72 hrs. RT storage showed a decline in WBC count using the optical but not the impedance method, resulting in a large number of WBC flags. An increase in mean corpuscular volume also was seen for patient specimens. The automated WBC differential showed a decrease in the percentage of neutrophils and an increase in the percentage of lymphocytes, owing primarily to neutrophil degeneration. These changes also were seen in the manual differential to a similar degree. Storage of specimens at 4°C largely prevented all of these changes. The implementation of refrigerated specimen storage is a simple, inexpensive method to improve the accuracy of CBC results for aged specimens on automated hematology analyzers.

KEYWORDS Haematology cell counter, CBC Count, Refrigeration, Platelets.

INTRODUCTION

All hematological tests begin with blood collection. To maintain good laboratory practice, it is essential to standardize pre-analytic procedures. These procedures consist of reception of test requests, registration of patient identification numbers, confirmation of test items, preparation of appropriate syringes and specimen containers, blood collection procedures, specimen storage and specimen transportation to the laboratory. Special attention must be paid to safe handling of blood [1]. Hematological testing must be performed on whole blood. Clinical laboratories equipped with modern automated analyzers are capable of processing large volumes of hematologic tests in an efficient and timely manner. These tests include complete blood count (CBC), differential leukocyte count (diff), reticulocyte count (retic), and more recently, the nucleated red blood cell count (NRBC).

The wide and cumulative knowledge concerning the behavior of CBC parameters over time under different storage conditions permits the identification of defined requirements and time thresholds for different applications in laboratory hematology, according to specific diagnostic demands. For instance, overnight storage of samples for routine CBC measurement may be acceptable, although predictable slight MCV increases and mild changes in the peripheral blood film can be present [2-3]. Similarly, refrigerated blood samples collected with EDTA can be used for internal instrument quality control for up to 3 days [3]. In more specific areas, such as blood testing for the anti doping athlete blood passport, that requires a forensic level of pre-analytical and analytical accuracy, robustness of the basic measures of hemoglobin and reticulocytes allows transport and analysis within 36 h of blood collected in any remote location, provided an efficient system of temperature control and monitoring is implemented [2-3].

The complete blood count (CBC) is a very common blood test. It evaluates the three major types of cells in blood: red blood cells (RBC), white blood cells (WBC), and platelets. These parameters are generally determined by an automated hematology analyzer that analyzes the different components of blood in a few minutes. The values generally included are the following: (1) WBC count - the number of white cells in a volume of blood, (2) WBC differential count – the relative numbers of the different types of WBC – usually classified into neutrophils, lymphocytes, monocytes, eosinophils, and basophils, (3) RBC count - the number of RBC in a volume of blood, (4) hemoglobin (Hb) - the amount of hemoglobin in a volume of blood, (5) hematocrit (Hct) - the ratio of the volume of RBC to the volume of whole blood, (6) mean corpuscular volume (MCV) - the average volume of a RB -, a calculated value derived from the hematocrit together with the RBC count, (7) mean corpuscular hemoglobin (MCH) - the average amount of hemoglobin in the average RBC, (8) mean corpuscular hemo globin concentration (MCHC) - the average concentration of hemoglobin in a given volume of RB -, a calculated volume derived from the hemoglobin measurement together with the hematocrit, (9) red cell distribution width (RDW) - a measurement of the variability of RBC size, and (10) platelets count - the number of platelets in the volume blood.

A increase or decrease in the number of a particular type of cells in the blood sample results will identify the health status. Many disease states are heralded by changes in the increase blood count, for example leukocytosis can be a sign of infection and thrombocytopenia can result from drug toxicity. For the hematological analysis, WBC count and morphological changes in these blood cell components occur after a long-term storage of the EDTA whole blood sample. The present study aimed to evaluate the storage time that the concentration of all blood parameters are stable or unstable blood parameters for 12,24,48 and 72 hrs in sysmax cell counter.

In the present study, sysmax cell counter was used in order to compare the stability of normal and pathological blood specimens collected in K2 EDTA tubes and stored at +4° C and +25° C up to 48 hours and evaluated if there is an advantage of storing at +4o C.

MATERIALS AND METHODS

A total of 100 blood samples from healthy volunteer (50 women and 50 men aged 18-60years) were obtained by vein puncture and samples collected in EDTA anticoagulated blood, specimens were processed through the sysmax cell counter at Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry. These subjects have given their informed consent. The blood samples were aliquot and were separated in to 2 parts, are kept at refrigerator temperature +4° C, other kept at +8° C and room temperature at +37° C. After collection of the blood samples an interval measurement was performed on sysmax cell counter haematology analyser. Subsequently, each sample was analysed for 6hrs, 12 hrs,48 hrs and 72 hrs.

The specimens stored at +4° C were allowed to equilibrate at room temperature for 30 minutes prior to analysis. Room temperatures (+37° C) were measured with a standard indoor thermometer and temperature follow up charts were daily filled as a part of quality control standards. The range of temperature during the study was +4° C, +8° C and +37° C.

RESULTS
Table 1: Comparison of Mean CBC parameters before and after storage of blood at room temperature

S.No.	Parameter	Initial	12 hrs	24 hrs	48 hrs	P Value
1	WBC(10^3/µl)	6.40±1.97	6.40±1.94	6.41±1.95	6.38±1.90	p>0.05
2	RBC(10^6/µl)	4.60±0.74	4.61±0.66	4.65±0.68	4.71±0.77	P<0.001
3	Hemoglobin(Hb)(g/dl)	14.05±2.18	14.01±2.11	14.01±2.11	14.09±2.18	P<0.05
4	Hemotocrit(Hct)(%)	40.50±5.60	40.50±5.51	41.75±5.58	41.88±5.67	P<0.001
5	MCV(µl^3)	86.64±8.26	88.41±8.40	90.30±8.51	96.43±8.50	P<0.001
6	MCH(pg)	29.89±3.68	30.31±3.77	30.40±3.79	36.44±8.61	P<0.05
7	MCHC(g/dl)	30.13±3.80	31.15±2.91	33.65±1.60	40.09±3.89	P<0.001
8	Platelet(Plt)(10^3/µl)	225.40±7.61	224.68±7.50	225.68±7.31	223.41±7.65	P>0.05

groups were compared for each parameter and percent changes were calculated. The values of the initial measurements were taken into account for comparisons. The percent change value obtained for each parameter was compared with the pre-determined imprecision value calculated for individual biological variation. Between groups differences were evaluated with paired Student's T test. Values of 'p' less than 0.05 were considered to be significant.

DISCUSSION
According to the recommendations of the Committee of International Haematology Standardization; Hematology analyzer manufacturers often quote that blood specimens kept at either at room temperature or at +4° C up to 24 hours generally reveal reliable results for complete blood count and automated differential count [4]. The recent trend towards large centralized laboratories have brought some obligations about the transfer and storage times of the blood specimens properly. Laboratories now test specimens that have been dispatched over a long distance; and the testing is often delayed by several hours or in some cases for days [5-6].

Previous studies have provided both supporting and conflicting results. [7] demonstrated sample stability after one week of incubation. Similarly, consistency was shown in RBC counts after 48 h and seven days of incubation at room temperature, respectively [8-9]. Contrastingly, [10] reported that RBC count decreased after 48 h at 37°C, while (10) reported WBC count stability for up to 3 days after blood collection. [11] incubated samples for 24 h and found significantly increased WBC counts.

Significant decrease of hemoglobin concentrations and RBC counts in blood samples stored at 33°C from BV was noticed after

60 hours from blood collection might be outcome of higher temperature induced conversion of some of the hemoglobin intermediates and due to significant autolysis of the RBCs associated with higher temperatures of storage [12].

Manufacturers of automated analyzers and published literature often state that blood specimens, kept at either room temperature or at +4o C (refrigerated) for up to 24 hours, generally reveal reliable results for Complete Blood Cell Count (CBC) [13-15]. However, these studies may not be satisfactory as the high variety of analyzers used are considered. Besides, no consensus was reached about which paremeters can still be reliable in delays over 24 hours when the analyzers were out of order due to different reasons [9,13-16]. In order to decide whether to accept or reject the aged specimen, laboratory staff needs to be familiar with the changes known to occur in blood specimens during storage [14].

Storage of samples at 4 °C – 8 °C for seven days increased the stability of most parameters. Parameters, namely WCC, platelet count, haematocrit, MCV and MCHC, as well as DIFF parameters, namely percentages of neutrophils and reticulocytes, were more stable when stored at 4 °C – 8 °C. However, some DIFF parameters, namely percentages of eosinophils, basophils and monocytes, had lower stability.

The stability of WBC and differentiation from automated haematology analyzers were slightly different depending on the principle used for detection, [2-3] reported that the stability of the WBC by Sysmex was 12 hr at RT. The change was granular and filaments were loose forming a pyknotic nucleus. [3] showed stability at 4°C for the WBC count, % neutrophil and % lymphocyte for 48 hr, while % monocyte and % eosinophil was stable for 72 hr. At RT the WBC count, monocyte and %eosinophil were stable for 24 hr, but %neutrophil and % lymphocyte were stable for only 12 hr. The results of our study were similar to previous study [2]. Showing refrigerated storage improved the stability of the blood cell count and differential WBC. At 4°C the WBC count was less stable than other parameters. The stability of these parameters was 72 hr, whereas the WBC count was 15 hr. However, 4°C was an appropriate storage temperature for the WBC count and differential WBC.

The parameters measured directly like WBC, RBC, hemoglobin and platelet remained stable for 2 days at room temperature as stated earlier in the study by previous study reported [9]. The degree of change observed in WBC count of both normal and pathological specimens throughout the study could be considered acceptable for clinical applications. However, a significant decrease in WBC and platelet count was observed when the same specimens were kept at + 4° C .

Hemoglobin concentration was found to be stable for the duration of the study. These findings are consistent with previous reports [9-10] changes in Hct in this experiment were consistent with that of other studies [9-10] reported that Hct was constant in different temperature but increased after 48 h increasing temperatures.

Unexpectedly we obtained negative percentage change in HTC and positive percentage change in MCV in normal specimens. But the situation which was found at the first 24 hours period measurement was not significant for both biological variation and statistically. This unsignificant change is considered as the calculations of Htc and MCV are in within run precision values. The increase in MCV in the specimens stored at room temperature reflects the swelling of RBCs. As also indicated by Wood et al. this increase is largely prevented by refrigerated storage [9,17]. In our study; we also concluded that the increase in MCV at room temperature was significant both biologically and statistically; whereas in refrigerated storage the increase in MCV was significant statistically while the target imprecision values for biological variation could not be passed. An increase in MPV is also observed besides MCV.

In the present study, MCV, MCH and MCHC all increased after 24-

h storage at room temperature. The increase in MCV is known to reflect red cell swelling at room temperature [10]. Our results of MCV and MCH were similar to previously reported results [9-10], although, there was no change in MCH for up to 4 days after collection of blood (de Baca et al., 2006). Additionally, it was reported that MCV of fresh blood in acid citrate dextrose (ACD) and EDTA was unchanged after storage for one day at 4°C but increased when the storage temperature was 23°C [18]. In our study, platelets counts were unchanged after 24-h incubation at room temperature. A previous study has demonstrated similar findings for up to four days incubation [9], while another contrastingly report showed increases in platelets counts after 48-h incubation and elevated temperature [10]. A study suggested that different temperatures and times of incubation can affect platelet counts and hemoglobin concentrations [19]. A study showed that the mechanism for the laboratory effect is that raising the temperature leads to changes in platelets morphology and movement [20].

Previous studies mentioned [4] the blood samples should not be left in the laboratory and testing should be conducted on blood samples as soon as possible, as delay in testing can lead to changes in certain CBC parameters.

But the increase in MPV was over the biological variation limits in specimens kept at refrigerator. The decrease in MCHC is caused by hematocrit increase when the hemoglobin level is still stable. As it is commonly known, MCHC is a parameter calculated by the ratio of hemoglobin to hematocrit multiplied by 100. Changes in the components of this equation will also lead to a change in MCHC value. But the changes detected in MCHC were not higher than the biological variation target values.

In this study, the percentage of reticulocytes was stable at RT until 12 hours after collection, which concurs with current recommendations [9-10]. However, the stability of reticulocyte parameters has been found to be longer at RT on sysmax haematology analysers.

In large academic laboratories, where aged samples make up a significant proportion of the workload, the storage time and temperature of samples must be taken into consideration. The findings of this study performed on EDTA samples add to the evidence that stability varies according to storage time and temperature.

According to the findings of this study, CBC parameters, namely RCC, haemoglobin, MCH and RDW, and DIFF parameters, namely percentages of basophils, lymphocytes and monocytes, were least affected by storage temperature and time and can be analysed until 48 hours after sample collection when stored at RT.

It is recommended that traditional CBC parameters be analysed 24 hours after sample collection when stored at RT [3-5]. However, in this study, platelets were only stable until 12 hours after collection when stored at RT. The stability of the WCC was also found to be shorter than other studies, which have recommended analysis up to 48 hours after collection when stored at RT. In this study, the WCC was stable only until 36 hours after collection when stored at RT.

In this study, the MCV was stable until 12 hours after collection. Previous studies [9-10] found that MCV increased significantly after 4–10 hours, regardless of the haematology analyser [5] whereas other studies have indicated a longer stability of up to 24 hours for MCV at RT. These discordant results may be attributed to the different statistical methods used in these studies for the evaluation of stability, which limits accurate comparison.

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 seven days are suitable for testing on the sysmax cell counter for FBC and reticulocyte parameters. However, this is not a solution for samples referred for DIFF or CBS morphology.

Based on the results, we concluded that the blood samples stored up to 72 hours at 4°C provide legitimate results for PCV, Hgb concentration and RBC count. MCV value is reliable if blood sample was stored up to 30 hours. Blood samples stored at 24 °C can be used for hemoglobin concentration and RBC count up to 72 hours of storage, but for PCV only up to 18 hours and MCV up to 12 hours from sample collection. Samples kept at 33°C give reliable results for MCV up to 12 hours, PCV up to 18 hours and hemoglobin concentration and RBC count up to 48 hours from blood collection.

This study can be used as a guide to determine the appropriate storage and handling of blood samples. In our study, it is obviously seen that especially the storage of pathological specimens at +4o C improves the stability when compared to normal specimens. As a result, we decided to increase the number of pathological specimens so as to make the changes more meaningful. The only stable parameters seem to be RBCs and hemoglobin if the measurements are carried out following a delay especially for the evaluations we made with the normal specimens. For other parameters; delayed processing affects stability. The delays and the possible effect of these delays on the results should be indicated in the reports. It is possible to maintain the stability of specimens by refrigerated storage up to two days with some limitations.

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