Reliability of Parameters of Complete Blood Count With Different Storage Conditions

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Introduction: The complete blood count (CBC) is a frequently performed laboratory test today. This study evaluated the effects of temperature and sample storage time on parameters of CBC which could produce misleading results of clinical significance. *Methods:* In a cross-sectional study, CBC was checked in 102 randomly selected healthy individuals and baseline measurements were analyzed using the Sysmex XS 500i fully automated hematology analyzer. CBC was done on samples up to 48 hr of storage at temperatures of $4 \pm 2°C$, $23 \pm 2°C$, and $31 \pm 2°C$. Values were checked at time intervals of 6, 24, and 48 hr. *Results:* Among CBC parameters, white blood cell, red blood cell, hemoglobin, mean cell hemoglobin (MCH), neutrophils and lymphocytes were stable at all three temperatures up to 48 hr. Monocytes, eosinophils, MCH concentration, hematocrit (Htc), and red cell distribution width-coefficient of variation showed statistically significant changes at $23 \pm 2°C$ and $31 \pm 2°C$. A significant decline in platelet count (PLT) and increment in mean platelet volume and basophil count were seen at all study temperatures up to 48 hr. *Conclusion:* This study shows that most parameters of the CBC are unaffected with the studied storage temperature up to 48 hr except for the PLT which should be performed within 6 hr of the post-collection time. To avoid changes in a few parameters such as Htc, it is best to store the sample at $4 \pm 2°C$ if any delay is anticipated. J. Clin. Lab. Anal. 31:e22042, 2017.

Key words: blood components; complete blood count; hematology; storage temperature; storage time

INTRODUCTION

Complete blood count (CBC) is one of the most commonly and routinely done laboratory tests today and this has become one of the first steps in the diagnostic work up in the clinical set up as it gives easy, valuable, and reliable information to the clinician not only in the diagnosis, but also in monitoring and prognosticating the patient. In many countries, centralized laboratories are equipped with modern automated analyzers that are capable of processing large volumes of hematological tests in an efficient and timely manner. Literature of most of the manufacturers of automated analyzers often cite that blood specimens kept at either room temperature (RT) or at $4 \pm 2°C$ (refrigerated) for up to 24 hr, generally yield reliable results for CBC and automated differential leukocyte count (1). The frequency of performing the CBC in tropical countries have tremendously increased due to the high incidence of conditions such as dengue which has a high mortality and morbidity. Due to this, a large number of specimens are transported for CBC analysis from collecting centers to a centralized laboratory where a delay of 1–3 days could occur. Therefore, it is vital to know the storage conditions of the sample. Storage at RT may cause ethylene diamine tetra acetate (EDTA) changes and quantitative effects of storage on blood as cellular elements are known to have limited stability in EDTA (2). The reliability of the hematocrit (Htc) and the platelet count (PLT) is of paramount importance in the management of the fatal

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dengue infection. As blood tests are commoner than testing other biological fluids, using standard methods for sample collection, incubation, and the role of environmental factors that affect the blood indices should be considered. When such a specimen arrives at the laboratory, the staff will need to decide whether to accept it or reject it; if accepted, whether to perform all of the ordered tests or only those appropriate based on the age of the specimen (1).

In this backdrop, this study was aimed at assessing the changes in various components of CBC with different storage temperatures at different incubation time periods as this would be helpful in determining which parameters can change with time and the conditions of the storage and the best temperature to store the sample, if a delay is anticipated.

MATERIALS AND METHODS

A total of 102 apparently healthy individuals, students of Faculty of Medical Sciences, University of Sri Jayawardenapura (USJP) USJP and some hospital staff members (age 20–50, male:female ratio 1:1) were randomly taken as participants in a descriptive cross-sectional study. Ethical approval was obtained from the Ethical review committee of USJP. Storage time and storage temperature were independent variables in this study. The three levels of storage temperatures were, RT (31 ± 2°C), refrigerated (4 ± 2°C), and air conditioned (AC) temperature (23 ± 2°C). Storage time had three levels, 6, 24, and 48 hr after sample collection. The dependent measure was the parameters of the CBC at specific storage time and the temperature. Each measurement was compared with the baseline measurement of the sample. K_2EDTA-anticoagulated blood was tested for CBC and report was issued to each individual. Several pre analytical variables such as age, gender, medications, and pregnancy were considered and those who were pregnant, on medication, who had chronic illness and who complained of any ailment were excluded (24 individuals). Blood specimens were processed through Sysmex XS-500i (Sysmax, Kobe, Japan) fully automated hematological analyzer at hematology clinic in colombo south teaching hospital (CSTH). Proper mixing of the sample was done prior to each analysis. Baseline measurement of the blood samples was performed at 20 min after the collection of the samples. After the initial measurement, each sample was divided into three portions. Thus, the three portions of each sample were stored at +4°C, AC temperature, and RT. The three temperatures were measured with a standard indoor thermometer and temperature follow-up charts were filled daily as a part of the quality control procedure. The temperatures were maintained at 31 ± 2°C (RT), 23 ± 2°C (AC), and 4 ± 2°C during the study. Following adequate mixing, all samples were reanalyzed after 6, 24, and 48 hr. Samples which were stored at 4°C were transferred immediately to the refrigerator after reanalyzing. Sysmex XS-500i (Sysmax), a five part hematology analyzer was used for sample analysis. This hematology analyzer uses fluorescent flowcytometric technology to give a precise measurement of white blood cell (WBC) count, red blood cell (RBC) count, and PLT. Hemoglobin (HGB) is measured using cyanide-free sodium lauryl sulfate method. Direct measurement of Htc is based on the total RBC count and the volume of each individual RBC. The CBC report consists of 24 parameters; total WBC, total RBC, HGB, Htc, mean cell volume (MCV), mean cell hemoglobin (MCH), MCH concentration (MCHC), red cell distribution width (RDW)-standard deviation and RDW-coefficient of variation (RDW-CV), PLT, platelet distribution width, mean platelet volume (MPV), plateletcrit (PCT), and the differential count include both absolute count and percentage of each leukocyte. In this study, we were interested only in 16 parameters out of 24. Calibration of the analyzer was performed using by the manufacturer. The daily quality control was performed using three levels (L1, L2, L3) of e-CHECK® (Sysmex corporation, Kobe, Hyogo, Japan) (XS) controls according to manufacturer instructions.

Statistical Analysis

Results were analyzed by the personal computer with SPSS Statistics for Windows, Version 17.0., SPSS Inc., Chicago, IL. Confidence level of 95 % was also determined using SPSS. Statistical significance of the differences between the mean values of each parameter was assessed by a paired sample *t*-test. $P < 0.05$ was considered statistically significant. Baseline value was standardized to 100, and mean percentage change was calculated for each parameter. The mean percentage change in each parameter which is between ±10% was taken as stable, whereas the mean percentage change higher than ±10% was considered as significant.

RESULTS

Descriptive cross-sectional study was done with blood samples taken from 102 healthy individuals aged between 20 and 50 years with a mean age of 25 years and the male female ratio was 1:1.

Storage Effects at 23 ± 2°C (AC Temperature)

At this temperature, WBC, RBC, HGB, and MCH were found to be stable up to 48 hr. MCV, RDW-CV,

and MPV increased with storage time and RDW-CV increased within 24 and 48 hr (Table 1). Statistically significant changes were found at 6, 24, and 48 hr of storage for RDW-CV and MPV and PLT. Underestimation of PLT occurred at any duration of storage. The mean percentage change in Htc was found to be higher than ±10% at both 24 and 48 hr (±17.3% and ±12.5%, respectively). The changes were statistically significant after 24 hr. The MCHC showed a downward trend with storage time up to 48 hr and statistically significant changes were seen at 24 and 48 hr of storage ($P < 0.05$). At this temperature, neutrophil, lymphocyte, and eosinophil counts were stable up to 48 hr, with a mean percentage change of less than ±10%. A decreased monocyte count was shown, which was not significant ($P > 0.05$). Statistically significant increment was found in basophil count after 24 hr of storage time.

Storage Effects at 4 ± 2°C (Refrigerated Temperature)

Prolonged storage of specimens for up to 48 hr at +4°C revealed the stability of CBC parameters, such as WBC, RBC, HGB, Htc, MCV, MCH, MCHC, and RDW-CV which were within ±10% of their original value and did not show any statistically significant changes (Table 2). PLT decreased over time which were statistically significant at 24 and 48 hr ($P < 0.05$) but stable at 6 hr. Statistically significant changes ($P < 0.05$) of increased MPV were observed at 24 and 48 hr. Neutrophil count was stable up to 24 hr and decreased during 48 hr of storage, where a statistically significant change was found at 48 hr of storage ($P < 0.05$). Out of the leukocytes, lymphocytes, monocytes, and eosinophils were stable over time with a mean percentage change less than ±10%. A statistically significant increment ($P < 0.05$) in basophil count was seen during the storage time of 6, 24, and 48 hr.

Storage Effects at 32 ± 2°C RT

WBC, RBC, HGB, MCH were stable up to 48 hr with a mean percentage change less than ±10%. PLT showed a significant decline with the time ($P < 0.05$), where the mean percentage change was higher than ±10% (−10%, −11%, −16.7%, respectively). MPV was found to be stable over time while Htc and RDW-CV were stable up to 6 hr and increased after 24 hr of storage. Statistically significant changes were seen in RDW-CV ($P < 0.05$). MCV and MCHC were found to be stable up to 6 hr and neutrophils and lymphocytes were found to be stable up to 48 hr. Twenty five out of 90 samples refused to give a neutrophil and a lymphocyte count at 48 hr of storage. Changes of monocyte and eosinophil counts started at 24 hr of

TABLE 1. Changes of CBC Parameter and WBC Differential Values Induced by Storage of Blood at 23 ± 2°C (AC Temperature)

	<1/2 hr Mean	6 hr Mean (CV%)	24 hr Mean (CV%)	48 hr Mean (CV%)
	$N = 102$			
WBC, $\times 10^9$/l	7.74	8.08 (104.3)	8.13 (105.3)	8.06 (104.1)
RBC, $\times 10^{12}$/l	4.47	4.56 (102.0)	4.52 (101.1)	4.52 (101.1)
HGB, g/l	12.59	12.84 (101.9)	12.80 (101.6)	12.78 (99.5)
Htc, l/l	37.01	37.75 (101.9)	43.42 (117.3)*	41.64 (112.5)*
MCV, fl	83.92	83.89 (100.0)	88.04 (104.9)*	92.81 (110.5)*
MCH, pg	28.63	28.61 (99.9)	28.72 (100.3)	28.77 (100.4)
MCHC, g/l	34.27	34.23 (99.8)	32.81 (95.7)*	30.91 (90.1)*
RDW % CV	13.14	13.50 (102.7)*	14.92 (113.54)*	14.93 (113.6)*
PLT, $\times 10^9$/l	277	252 (90.9)*	242 (87.3)*	253 (91.3)*
MPV	9.99	10.40 (104.1)*	10.84 (108.5)*	11.23 (112.4)*
Neutrophil, $\times 10^9$/l	4.03	4.30 (106.6)	4.35 (107.9)	4.14 (102.7)
Lymphocyte, $\times 10^9$/l	2.75	2.80 (101.8)	2.85 (103.6)	2.74 (99.6)
Monocyte, $\times 10^9$/l	0.61	0.61 (100.0)	0.51 (83.6)	0.55 (90.1)
Eosinophil, $\times 10^9$/l	0.32	0.34 (106.2)	0.35 (109.3)	0.33 (103.1)
Basophil, $\times 10^9$/l	0.03	0.04 (133.3)*	0.05 (166.6)*	0.08 (266.6)*

The data were presented as means of parameter values, changes (% from original value, which is 100%) at different time points and SDs of changes in parenthesis.

AC, air conditioned; CBC, complete blood count; HGB, hemoglobin; MCH, mean cell hemoglobin; MCHC, MCH concentration; MCV, mean cell volume; MPV, mean platelet volume; PLT, platelet count; RBC, red blood cell; RDW-CV, red cell distribution width-coefficient of variation; WBC, white blood cell; Htc, hematocrit.

*Statistically significant at 0.05 level.

TABLE 2. Changes of the CBC Parameters Induced by Storage of Blood at 4 ± 2°C (Refrigerated Temperature)

	<1/2 hr Mean	6 hr Mean (CV%)	24 hr Mean (CV%)	48 hr Mean (CV%)
	$N = 102$			
WBC, $\times 10^9$/l	7.74	8.01 (103.4)	7.79 (100.6)	7.16 (92.5)
RBC, $\times 10^{12}$/l	4.47	4.59 (102.6)	4.56 (102.0)	4.56 (102.0)
HGB, g/l	12.59	12.92 (102.6)	12.88 (102.3)	13.88 (110.2)
Htc, l/l	37.01	37.39 (101.0)	37.45 (101.1)	37.46 (101.2)
MCV, fl	83.92	82.52 (98.3)	82.70 (98.5)	83.17 (99.4)
MCH, pg	28.63	28.64 (100.0)	28.67 (100.1)	28.86 (100.7)
MCHC, g/l	34.27	34.87 (101.7)	34.87 (101.7)	34.87 (100.7)
RDW % CV	13.14	13.16 (100.1)	12.99 (98.8)	13.00 (98.9)
PLT, $\times 10^9$/l	277	270 (90.2)	240 (86.6)*	231 (83.3)*
MPV	9.99	10.01 (99.1)	11.02 (110.3)*	11.27 (112.8)*
Neutrophil, $\times 10^9$/l	4.03	4.30 (106.6)	4.08 (101.2)	4.2 (104.6)
Lymphocyte, $\times 10^9$/l	2.75	2.74 (99.6)	2.70 (98.1)	2.62 (95.2)
Monocyte, $\times 10^9$/l	0.61	0.65 (106.5)	0.63 (103.2)	0.57 (93.4)
Eosinophil, $\times 10^9$/l	0.32	0.33 (103.1)	0.35 (109.3)	0.34 (106.2)
Basophil, $\times 10^9$/l	0.03	0.04 (133.3)*	0.06 (200.0)*	0.10 (333.3)*

The data were presented as means of parameter values, changes (% from original value, which is 100%) at different time points and SDs of changes in parenthesis.

CBC, complete blood count; HGB, hemoglobin; MCH, mean cell hemoglobin; MCHC, MCH concentration; MCV, mean cell volume; MPV, mean platelet volume; PLT, platelet count; RBC, red blood cell; RDW-CV, red cell distribution width-coefficient of variation; WBC, white blood cell; Htc, hematocrit.

*Statistically significant at 0.05 level.

storage ($P < 0.05$). Basophil count increased with time which was statistically significant at 6, 24, and 48 hr of storage ($P < 0.05$).

DISCUSSION

In this study, the storage effects of parameters of CBC at different storage temperatures at different post collection intervals were analyzed.

According to the recommendations of the International Committee of Haematology Standardization and reviews following that the maximum storage intervals for CBC and WBC count with automated differential count is stable at 4°C for at least 24 h, or even to 72 h, with significant differences depending on the type of automated blood cell analyzer. However, this becomes a difficult task as there are large numbers of samples transported from peripheral collecting centers to the central laboratories, sometimes with a delay of more than 24 hr. Therefore, it is important to decide about the suitable duration of storage and the temperature, if a delay of analysis is anticipated. Refrigerated storage of EDTA-anticoagulated blood has been noted to improve the stability of CBC (1–7).

Previous studies provide supporting and conflicting results regarding the findings of WBC, RBC, and HGB, where our study revealed that these parameters were constant at all temperatures throughout the study. Most surveys which have been done at RT reported that WBC, RBC, and HGB were constant throughout the duration of the study which are similar to our study results (1, 4–7). However, a few studies which were done using other temperatures such as refrigerated temperature revealed that WBC and HGB were constant at refrigerated temperature up to 3 days (3, 4).

In our study, Htc showed significant changes at RT and AC temperature but stable at 4°C which is similar to the many other studies (1, 6–8).

This becomes important in monitoring dengue patient in the leaking phase with Htc as many wards have a higher temperature above 25°C in most settings in tropical Sri Lanka.

MCV was found to be stable at 4°C up to 48 hr and showed significant changes after 24 hr at RT and at AC temperature. MCV also reflected a significant increment up to 24 hr and decrease in 48 hr. Previous studies also revealed supporting results regarding MCV which are similar to our findings (1, 2, 5, 7, 8). Finding of MCV at 4°C in Turan et al. (2) demonstrated that MCV was stable on the first day but an increment was observed on the second day. The increment of MCV reflected the swelling of the RBC at RT. Hence, the increment in Htc with relatively stable HGB leads to a steady decline in MCHC over time (2). MCHC was found to be stable at 4°C over time, but decreased at AC temperature and revealed significant changes at RT when stored for 24 hr. Most of

the studies showed similar results to our study at RT but Nargeset et al. (7) demonstrated that MCHC significantly increased at RT.

MCH was found to be stable at all three temperatures over time. It is supported by previous studies which were done at RT (1, 5–8). However, contradicting to this, Turan et al. (2) demonstrated that MCH increased at 4°C over time.

Our study revealed that RDW increased significantly from 24 hr of storage at RT and AC temperature. The increased MCV could be the possible cause for this change. Similar findings were seen in other studies. (1, 5, 8).

One of the most important findings in our study was the statistically significant decline in PLT at all temperatures from 6 hr onwards. Some studies have shown that the PLT were stable even up to 24 hr if they were stored at 4°C (9), where as other studies showed that the PLT tend to increase during incubation due to its shape change and fragmentation (6). The increment of MPV was seen at all temperatures within the duration of the study. Many studies show that MPV increases over time (1, 2, 4, 5, 8). It is possible that the PLTs which show an increment of the MPV will not be counted as platelets by the machine but be flagged separately leading to a reduction in the PLTs. This shows that it is best to assess PLT within 6 hr of collection. A few studies showed that a higher temperature leads to changes in PLT morphology and movement, decreasing the count which can be minimized by storing it at a lower temperature (10, 11).

Our findings of the automated differential count for specimens stored at 4°C revealed minimum changes of absolute differential count compared to the RT and AC temperatures. At 4°C, the absolute counts of neutrophil, lymphocyte, eosinophil, and monocyte were stable over the time period except for the basophil count. Absolute count of basophil increased with time. Significant changes were observed in monocyte, eosinophil, and basophil counts at RT. Neutrophil, eosinophil, monocyte, and basophil counts reflected significant changes, whereas lymphocyte count was stable at AC temperature. Previous studies of Gulati et al. and Vogelaar et al. (1, 4) also reported that significant changes arise in WBC differential parameters at RT over time. Hedberg and Lehto (8) demonstrated that WBC differentials were stable at 48–72 hr, with a slight decrease observed in absolute neutrophils and lymphocytes at 4°C.

Many studies done on effects of storage conditions on CBC parameters at different storage conditions have revealed specific requirements for pre-analytical specimen storage depending on the parameters required for purposes of diagnosis and management (12). The storage effects on most of the blood count parameters can be minimized if the storage temperature is maintained at 4°C and if the analysis takes place within 24 hr (3, 12). Similar data were found in other studies, where it was found that the refrigeration of samples (4–6°C) minimized the variability in the CBC parameters (9).

CONCLUSION

According to results and the review of literature, it is preferable to perform CBC and differential count on blood specimens as soon as possible following venipuncture. The only parameters which are reliable at the given temperatures and the duration of storage up to 2 days are WBC, RBC, HGB, and MCH.

Reduction of PLT with the storage time at any given temperature, other than when stored at 4°C up to 6 hr was an important finding in our study.

Decisions such as hospital admission and recovery are often decided on the PLT count and the Htc in illnesses such as dengue fever. Other parameters such as MCV, MCHC, MPV, and some cells of the differential count other than neutrophils and lymphocyte are subjected to changes with storage at different temperatures. Therefore, it is preferable to indicate not only the time and the date of the sample collection but also the time and date of sample analysis. According to the results of our study, CBC parameters are more likely to change in temperate climate, and caution should be taken to store them at 4°C when a delay is anticipated over 6 hr, where other than PLT and basophils, all other parameters are stable up to 48 hr.

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