

Predictive WBC Count and Differential WBC on Time Variation

Orathai Tangvarasittichai, Ph.D., Surapon Tangvarasittichai, Ph.D.

Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand.

ABSTRACT

Objective: To evaluate the storage time that the concentration of all blood parameters are stable and to propose the predictive formula for the unstable blood parameters.

Methods: EDTA blood samples from 22 healthy volunteer subjects were aliquot and separated into 2 parts, one kept at refrigerator temperature (4°C) and the other kept at room temperature (RT). Complete blood counts (CBC) were determined in each blood sample with a CELL DYN 3200 hematology analyzer every hour for 24 hours and every 2 hours from 26 to 72 hours.

Results: The RBC count, RBC indices, platelet count, and differential WBC were stable for 72 hr blood storage at 4°C except the WBC count was decreased after 15 hr storage. The predictive WBC count after 15 hr storage at 4°C was $WBC_0 = WBC_T + 0.081(T-15)$.

Conclusion: This study establishes the predictive formula for the initial time value which is useful for laboratories that have a problem with instrument failure or for mobile units that cannot measure CBC immediately.

Keywords: Complete blood count, CELL DYN 3200, predictive value, stability

Siriraj Med J 2008;60:248-250

E-journal: <http://www.sirirajmedj.com>

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 RBC -, 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 RBC -, a calculated value 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. The CBC results determine the health status that is defined by an absolute increase or decrease in the number of a particular type of cells in the blood sample. 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 and to propose the predictive formula for the unstable blood parameters.

MATERIALS AND METHODS

Blood samples from healthy volunteer 22 subjects (11 women and 11 men, aged 19-60 years), were

Corresponding to: Orathai Tangvarasittichai
E-mail: Orathai19t@yahoo.com

TABLE 1. The stability of parameters at room temperature and 4°C from 22 samples.

Test	Concentration		Stability (hour)	
	Mean	% CV	RT	4°C
WBC count	7.5 x10 ⁹ cells/L	9.8	0 - 15	0 - 15
% neutrophil	52.0 %	12.2	0 - 15	0 - 72
% lymphocyte	36.5 %	11.4	0 - 15	0 - 72
% monocyte	8.1 %	18.2	0 - 18	0 - 72
% eosinophil	3.3 %	24.4	0 - 12	0 - 72
% basophil	1.0 %	20.1	0 - 24	0 - 72
RBC parameters	5.2 x10 ¹² cells/L	7.5	0 - 72	0 - 72
Platelet count	152 x10 ⁹ cells/L	11.4	0 - 72	0 - 72

obtained by venipuncture and stored in EDTA tubes (1.5 mg/ml of blood). These subjects have given their informed consent. The blood samples were aliquot and were separated into 2 parts, one kept at refrigerator temperature (4°C) and the other kept at room temperature (28°C). Immediately after blood sampling, an initial measurement was performed on the CELL DYN 3200 haematology analyzer. Subsequently, each sample was analysed every hour for 24 hours and every 2 hours from 26 to 72 hours.

CELL DYN 3200 Haematology Analyzer

The CELL DYN 3200 Haematology Analyzer (Abbott Laboratories, USA) is an automated haematology analyzer with a five part differential screening device. White blood cell differentiation is performed by a multi-angle polarized scatter separation method.

Data analysis

The stability of the parameters was analysed by repeated measurement ANOVA and multiple comparison by least-significant different (LSD) from the 22 samples with a variation in time, whereas the predictive formulas were estimated by regression analysis. All statistics were performed by SPSS program. The statistical significance is P-value < 0.05.

RESULTS

The stability study of WBC, RBC and platelet parameters in EDTA blood samples was evaluated at room temperature (RT) and 4°C storage samples for 72 hr. These samples were performed by a CELL DYN 3200

analyzer. The blood component parameters stored at RT and 4°C showed a statistically significant decrease in WBC count after 15 hr (Table 1). Fig 1 represents the WBC count in the monitoring times and was not significantly different at 0-15 hr by ANOVA (P-value= 0.85) at both temperatures. The differential WBC count, %neutrophil, %lymphocyte, %monocyte, %eosinophil, and %basophil were changed only in blood samples stored at RT. There were significant sequential decreases of %neutrophil and %eosinophil after 15 and 12 hr, respectively at RT (Fig 2). Whereas %lymphocyte, %monocyte and %basophil were changed after 15, 18 and 24 hr, respectively (Fig 3). At 4°C, the WBC differential count was not changed for 72 hr. The results of the EDTA blood storage samples in each time of the measurement suggested that the change in each blood component parameter was linear varying by time. Then, we generated the estimation formula for each parameter as shown in Table 2 in which the formulas were calculated by the linear regression equation. Hence the 4°C blood storage sample is recommended, since only the WBC count was changed after 15 hr. If the EDTA blood sample cannot immediately be analysed and is stored at 4°C over 15 hr, the WBC count can be corrected by $WBC_0 = WBC_T + 0.081(T-15)$. Using this formula, we can obtain the corrected values of each blood cell parameters after a period of storage-time.

DISCUSSION

The stability of WBC and differentiation from automated haematology analyzers were slightly different depending on the principle used for detection. Boos, et al. and Bartels, et al. 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. Sachsec C, et al. 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 %lym-

TABLE 2. The predictive formula parameters with time dependent changes.

Test	Temperature of blood sample storage		R ²	4°C	R ²	SEE
	RT					
WBC count ₀	$WBC_0 = WBC_T + 0.037(T-15)$		70%	$WBC_0 = WBC_T + 0.081(T-15)$	81%	0.11
% neutrophil ₀	$\% neu_0 = \% neu_T + 0.824(T-15)$		74%	stable	-	
% lymphocyte ₀	$\% lym_0 = \% lym_T - 0.329(T-15)$		40%	stable	-	
% monocyte ₀	$\% mono_0 = \% mono_T - 0.398(T-18)$		50%	stable	-	
% eosinophil ₀	$\% eo_0 = \% eo_T + 0.073(T-12)$		77%	stable	-	
% basophil ₀	$\% baso_0 = \% baso_T - 0.173(T-24)$		52%	stable	-	

WBC₀ = WBC at initial time

WBC_T = WBC at time of measurement after 15 hr

For example at 20 hr storage at 4°C, the WBC count was 4.0 x10⁹ cells/L, so at initial time the WBC count was 4.0+0.08(20-15) = 4.4 x10⁹ cells/L

R² = Coefficient of determination

SEE = Standard error of the estimation

Stable is the parameter was no significant mean difference for 72 hr.

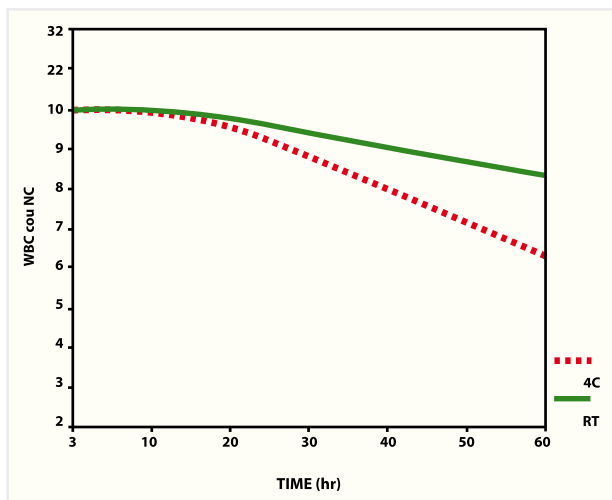


Fig 1. The different mean of the WBC count at 4°C and RT. The WBC count was no significant difference at 0-15 hr ($p < 0.05$) in both temperatures.

phocyte were stable for only 12 hr. The results of our study were similar to Brent L. Wood, et al. 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

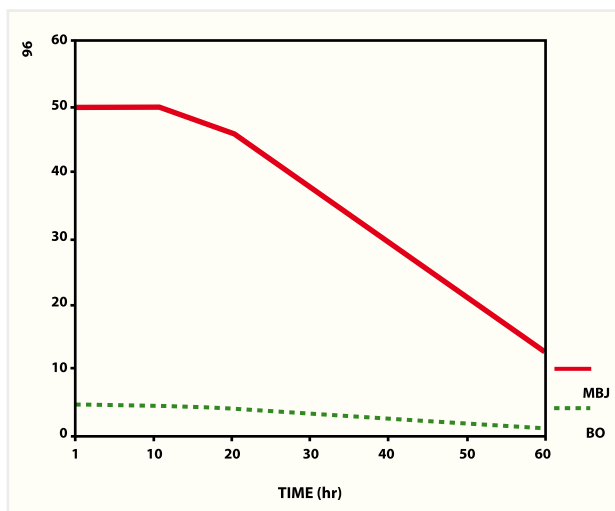


Fig 2. The stability of the %neutrophil (neu) and %eosinophil (eo) count at RT. The %neutrophil and eosinophil count was decreased after 15 hr storage at RT.

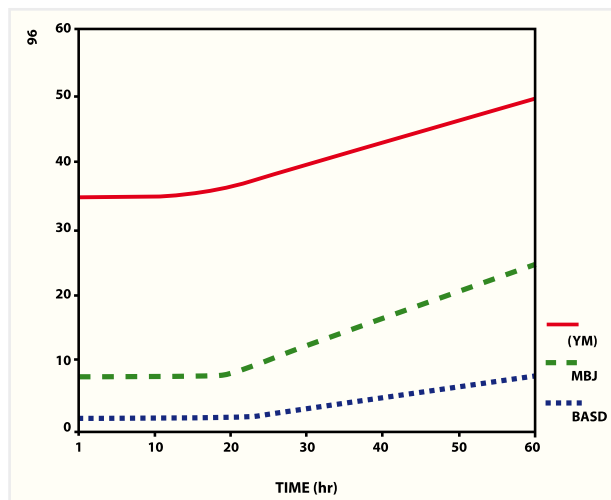


Fig 3. The stability of the %lymphocyte (lym), %monocyte (mono) and %basophil (baso) count at RT. The %neutrophil and eosinophil count was increase after storage at RT.

storage temperature for the WBC count and differential WBC. Furthermore, this study suggested the predictive formula, $WBC_0 = WBC_T + 0.081 (T-15)$ for the WBC count after 15 hr. Hence, it is advantageous to laboratories that have problems with malfunctioning hematology analyzers and cannot use fresh some samples. It is also useful for analyzing and ensuring the accuracy of samples from mobile units at far away places.

ACKNOWLEDGMENTS

This study was supported by the Faculty of Allied Health Sciences, Naresuan university. The author would like to thank Assoc. Prof. Dr. Mary Sarawit for her help in preparing the manuscript.

REFERENCES

1. Bartels PCM, Schoorl M. Time dependent increase of differential monocyte count on the Sysmex NE-8000. Clin Lab Haem 1998;20:165-8.
2. Boos MS, Stewart RE, Miller ML. Temperature and storage dependent changes in hematologic variables and peripheral blood morphology. Am J Clin Pathol 1998;110:537.
3. Sachsec C, Jahn-Streurelg G, Henkel E. First clinical evaluation of the CELL-DYN3200 haematology analyzer. Clin Lab Haem 1998;20:333-40.
4. Wood BL, Andrews J, Miller S, Sabath DE. Refrigerated storage improves the stability of the complete blood cell count and automated differential. Am J Clin Pathol 1999;12:687-95.