Introduction

In clinical laboratories, the cycles of each test start with the preparation of patient, and continue with biologic sample collection, preparation and incubation of the sample in suitable conditions and finally finish by reporting the result. It is sometimes necessary to repeat a test to obtain a reliable result ***(Vogelaar et al., 2002)***. Sometimes there is a period of time between sample collection and doing or redoing a test. In this situation the stability of the sample is very important. Since blood tests are more common than other biological fluids tests, therefore using the standard methods for sample collection, incubation and the role of environmental factors that affect the blood indices should be considered ***(Mahmoodi et al., 2006)***.

Complete blood count (CBC) is one of the most common and routine laboratory tests that is the first step to diagnose an illness and since this test becomes easy and quick, it can give valuable information to the physicians. The results of CBC can be affected by different factors such as the temperature and incubation period ***(Mahmoodi et al., 2006)***.

However, it is not uncommon for the laboratories to receive specimens that are 1 to 3 days old. Furthermore, it is a common practice to keep these specimens at room temperature until delivery to the laboratory and processing ***(Bennett-Guerrero et al., 2007)***. Manufacturers of automated analyzers and published literature usually cite that blood specimens kept at room temperature for 24 hours to 48 hours generally yield reliable results. However, specific information concerning the suitability or unsuitability of specimens older than 1 or 2 days for various automated hematologic tests is limited ***(De Beca et al., 2006)***.

Room temperature storage showed a decline in white blood cell (WBC) count using the optical but not the impedance method, resulting in a large number of WBC flags ***(Hirase et al., 1992)***. At room temperature the WBC count, percentage monocytes and percentage eosinophils were stable for 24 hour, but percentage neutrophils and percentage lymphocytes were stable for only 12 hours ***(Tangvarasittichai and Tangvarasittichai, 2008)***. In addition, several studies determined that the long-term incubation of blood in room temperature changes some blood indices ***(Gulati et al, 2002)***. Storage of specimens at 4 degree Celsius (°C) largely prevented all of these changes ***(Davies et al, 1997)***.

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 ***(Wood et al., 1999)***. Several studies showed at

4°C, WBC count showed stable percentage neutrophils and percentage lymphocytes for 48 hour, while percentage monocytes and percentage eosinophils were stable for 72 hour ***(Tangvarasittichai and Tangvarasittichai, 2008)***.

Aim of the Work

The aim of this study is to evaluate the changes that occur in various parameters of automated CBC and differential count during storage of blood at room temperature for 6 days as well as in the refrigerator.

Complete Blood Count

The CBC is a basic screening test and one of the most frequently ordered laboratory procedures. The findings in the CBC give valuable diagnostic information about the hematologic and other body systems, prognosis, response to treatment, and recovery. The CBC consists of a series of tests that determine number, variety, percentage, concentrations, and quality of blood cells ***(Fischbach and Dunning, 2009)***.

Definitions of Hematology Parameters

The following hematology parameters are reported for a typical CBC and may be flagged for a value outside the normal range:

- **Red blood cell (RBC) count** is the number of RBCs per microliter (µL) of blood. An elevated RBC can reflect polycythemia (reactive or neoplastic) or a disorder of globin synthesis. A decreased RBC typically reflects anemia.

- **Hemoglobin (Hb)** is the concentration of Hb in whole blood, in gram/deciliter (g/dl). An increased Hb may reflect a polycythemia (reactive or neoplastic) or be due to dehydration. A decreased Hb typically reflects anemia.

- **Hematocrit (HCT)** is the packed spun volume of blood made up of RBC, expressed as a percentage of total blood volume. It can be measured or calculated as HCT = (RBC x Mean Corpuscular Volume (MCV)/10. An increased HCT can reflect polycythemia (reactive or neoplastic); if calculated, an increased HCT may reflect a normal number of RBC with an elevated MCV.

- **Mean corpuscular volume** is the average volume (size) of the patient's RBCs. It can be measured or calculated as MCV in femtoliter (fL) = 10 x HCT (percent (%) ÷ RBC (millions/ µL). Anemia can be classified based on whether the MCV is low, normal, or elevated.

- **Mean corpuscular hemoglobin (MCH)** is the average Hb content in a RBC It is calculated as MCH (picogram (pg)/red cell) = Hb (g/dl) x 10 ÷ RBC (millions/µL). A low MCH indicates decreased Hb content per cell, and is typically reflected in hypochromia on the peripheral blood smear. This may be seen in iron deficiency and disorders of globin synthesis.

- **Mean corpuscular hemoglobin concentration (MCHC)** is the average Hb concentration per RBC, in g/dl. It is calculated as MCHC (g/dl) = Hb (g/dl) X

100 ÷ HCT (%). Low and high MCHC values are helpful in classifying anemia. Very low MCHC values are typical of iron deficiency anemia, and very high MCHC values typically reflect spherocytosis or RBC agglutination. Examination of the peripheral blood smear is helpful in distinguishing these findings.

- **Red cell distribution width (RDW)** is a measure of the variation in RBC size, which is reflected in the degree of anisocytosis on the peripheral blood smear. A high RDW implies a large variation in RBC sizes, and a low RDW implies a more homogeneous population of RBCs. RDW is calculated; the RDW is the coefficient of variation (CV) of the RDW = (standard deviation (SD)/MCV) x 100. RDW is helpful in the classification of anemia. A very elevated RDW can be seen in iron deficiency, transfused anemia, myelodysplastic syndrome, and homozygous hemoglobinopathy, whereas slightly elevated RDW can be seen in thalassemia trait and anemia of chronic disease.

- **White blood cell count** is the number of WBC per µL of blood. An elevated WBC (ie, leukocytosis) may be seen in neoplastic and non-neoplastic conditions. If the WBC is elevated, enumeration of the WBC differential and review of the peripheral blood smear is used along with clinical evaluation to determine the cause.

If the WBC is decreased (ie, leukopenia), a WBC differential should be obtained and the peripheral blood smear examined to enumerate which cell type is decreased. Since neutrophils make up the greatest percentage of WBCs, leukopenia is usually due to neutropenia at a minimum (and perhaps decreases in other WBCs as well).

- **Platelet (PLT) count** is the number of PLT per µL of blood. An elevated PLT count (i.e., thrombocytosis, also called thrombocythemia) may be seen in reactive and neoplastic conditions. A decreased PLT count (i.e., thrombocytopenia) may reflect PLT destruction, sequestration, or ineffective thrombopoiesis. Examination of the peripheral blood smear is helpful in distinguishing among possible causes.
- **Mean platelet volume (MPV)** is the average volume (size) of the patient's PLTs. Evaluation of the MPV should be done in context of the PLT count ***(George, 2014).***

Normal Values of Various Parameters of CBC:

Complete blood count varies by age, sex, race and demography. Normal values (reference ranges) are ultimately determined by the laboratory performing the test in the particular population. As a guide, the normal values for adult men and women are as shown in **table 1**:

Table 1: Normal adult values (reference ranges) of CBC parameters.

RBC Parameters	
• Red Blood Cell Count 3.8-4.8 × 10^{12}/Liter(L) for women • Hemoglobin 12.0 – 15.0 g/dl for women • Hematocrit 40.5 – 41.5 % for women • Mean Corpuscular Volume 83 – 101 fL • Mean Corpuscular Hemoglobin 27 – 32 pg • Mean Corpuscular Hemoglobin Concentration 31.5 – 34.5% • Red Cell Distribution Width -CV) 11.6-14.0% • Red Cell Distribution Width -SD) 39-46fL	• 4.5 – 5.5 × 10^{12}/L for men • 13.0 – 17.0 g/dl for men • 44.5 – 45.5% for men
WBC Parameters	
Total WBCs: 4.000-10.000×10^{9}/L for women and men • Neutrophils (40-80 %) 2.0-7.0×10^{9}/L • Lymphocytes (20-40 %) 1.0-3.0×10^{9}/L • Monocytes (2-10 %) 0.2-1.0×10^{9}/L • Eosinophils (1-6 %) 0.02-0.5×10^{9}/L • Basophils (<1-2 %) 0.02-0.1×10^{9}/L • Bands 0-5%	
PLT Parameters	
• Platelets 150.000-410.000×10^{9}/L • Mean Platelet Volume 7.4-10.4 fL • Platelet Distribution Width 9-13 fL • Plateletcrit 0.108-0.282 %	

(Bain et al., 2012)

Samples:

A phlebotomist collects the sample, drawing the blood into a test tube containing an anticoagulant Ethylene diamine tetra- acetic acid (EDTA) sometimes citrate, to stop it from clotting. The sample is then transported to a laboratory. Sometimes the sample is drawn off a finger prick using a Pasteur pipette for immediate processing by an automated counter ***(Napolitano et al., 2009)***.

Physiological Variations in the Blood Count

(I) Red cell components

There is considerable variation in the RBC count and Hb concentration at different periods of life and there are also transient fluctuations, the significance of which is often difficult to assess. At birth the Hb is higher than at any period subsequently. The RBC is high immediately after birth, and values for Hb >200 g/L, RBC higher than 6.0 - 10/L and a HCT over 0.65 are encountered frequently when cord clamping is delayed and blood from the placenta and umbilical artery re-enter the infant's circulation. After the immediate postnatal period, the Hb falls fairly steeply to a minimum by about the 2nd month. The RBC and HCT also fall, although less steeply, and the cells may become microcytic with the development of iron deficiency ***(Bain et al., 2012)***. The Hb and RBC increase gradually through childhood to reach almost adult levels by puberty ***(Taylor et al., 1997)***.

A. Pregnancy

In normal pregnancy, there is an increase in erythropoietic activity. However, at the same time, an increase in plasma volume occurs, and this results in a progressive decrease in Hb, HCT and RBC **(Table 2)**. The level returns to normal about a week after delivery. There is a slight increase in MCV during the 2nd trimester. Serum ferritin decreases in early pregnancy and usually remains low throughout pregnancy, even when supplementary iron is given ***(McMullin et al., 2003)***.

Table 2: Hb concentration values in pregnancy	
• 1st Trimester	124-135 g/L
• 2nd Trimester	110-117 g/L
• 3rd Trimester	106-109 g/L

(Bain et al., 2012)

B. The elderly

In healthy men and women, Hb, RBC, HCT and other red cell indices remain remarkably constant until the 6th decade. Anemia becomes more common in those older than 70–75 years and is associated with poor clinical outcomes due to poorer cognitive status, increased frailty and an elevated risk of hospitalization and of complications during hospitalization. In the elderly, the difference in Hb between men and women is less than in younger subjects, so that a difference of 20 g/L in younger age groups is reduced to 10 g/L or less in the elderly. There is a concomitant increase in serum iron in women, although serum ferritin levels remain higher in men than in women ***(Steensma and Tefferi, 2007)***.

C. Exercise

Optimal athletic performance depends on proper function of many organs, including the blood. Several hematological parameters can affect or be influenced by physical activity including blood cells and coagulation mechanisms. For example endurance athletes may develop so-called sports anemia', which is thought to be the result of increased plasma volume. Increasing oxygen delivery by raising the HCT is a simple acute method to improve athletic performance. Legal means of raising the HCT include altitude training and hypoxic tents. Illegal means include blood doping and the administration of erythropoietin (EPO). Endurance athletes may also have decreased levels of serum iron and ferritin, possibly associated with loss of iron in sweat. Conversely, in sprinters who require a short burst of very strenuous muscular activity, there is a transient increase in RBC by 0.5×10^{12}/L and in Hb by 15 g/L, largely because of reduction in plasma volume and to a lesser extent the re-entry into the circulation of cells previously sequestered in the spleen ***(Cooper, 2008)***.

D. Smoking

Both active and passive cigarette smoking have a significant effect on many hematological normal reference values. Some effects may be transient and their severity varies between individuals as well as by the number of cigarettes smoked. Smoking ≥ 10 cigarettes a day results in slightly higher Hb, HCT and MCV. This is probably at least in part a consequence of the accumulation of carboxy-haemoglobin in the blood together with a decrease in plasma volume. After a single cigarette, the carboxyhaemoglobin level increases by about 1%, and in heavy smokers the carboxyhaemoglobin may constitute 4–5% of the total Hb Smoking may be associated with polycythaemia ***(Andrews and Tingen, 2006)***.

(II) Leucocyte count

At birth, the total leucocyte count is high; neutrophils predominate, reaching a peak of 13.0 × 10^9/L within 6–8 hours for neonates of >28 weeks' gestation and 24 hours for those delivered at <28 weeks. The count then falls to 4.0×10^9/L over the next few weeks and then to a level at which the count remains steady. The lymphocytes decrease during the first 3 days of life often to a low level of 2.0-2.5 × 10^9/L and then rise up to the 10^{th} day; after this time, they are the predominant cell (up to about 60%) until the 5^{th} to 7^{th}

year, when they give way to the neutrophils. From that age onwards, the levels are the same as for adults. There are also slight sex differences; the total leucocyte count and the neutrophil count may be slightly higher in girls than in boys and in women than in men. After the menopause, the counts fall in women so that they tend to become lower than in men of similar age. People differ considerably in their leucocyte counts. Some tend to maintain a relatively constant level over long periods; others have counts that may vary by as much as 100% at different times. In some subjects, there appears to be a rhythm, occurring in cycles of 14–28 days and in women this may be related to the menstrual cycle or to oral contraception ***(Karandikar et al., 2002)***.

Random activity may raise the count slightly; strenuous exercise causes increases of up to 30×10^9/L, partly because of mobilization of marginated neutrophils and changes in cortisol levels. Large numbers of lymphocytes and monocytes also enter the bloodstream during strenuous exercise. However, there have also been reports of neutropenia and lymphopenia in athletes undergoing daily training sessions ***(Watson and Meiklejohn, 2001)***.

A moderate leucocytosis of up to 15×10^9/L is common during pregnancy, owing to a neutrophilia, with the peak in the 2^{nd} trimester. The count returns to nonpregnancy levels a week or so after delivery. Cigarette smoking has an effect on the leucocyte count the leucocyte count increases, largely as a result of an increase in the neutrophils and neutrophil function may be affected. Smoking may also cause an increase in CD4-positive lymphocytes and total lymphocyte count ***(Karandikar et al., 2002)***.

(III) Platelet count

There is a slight diurnal variation in the PLT count of about 5%; this occurs during the course of a day as well as from day-to-day. Within the wide normal reference range, there are some ethnic differences and in healthy West Indians and Africans PLTs counts may on average be 10–20% lower than those in Europeans living in the same environment. There may be a sex difference; thus, in women, the PLT count has been reported to be about 20% higher than in men. A decrease in the PLT count may occur in women at about the time of menstruation. There are no obvious age differences; however, in the 1st year after birth the PLT count tends to be at the higher level of the adult normal reference range. Strenuous exercise causes a 30–40% increase in PLT count; the mechanism is similar to that which occurs with leucocytes ***(Avloniti et al., 2007)***.