

**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF MEDICINE
DEPARTMENT OF MEDICAL LABORATORY
SCIENCES**



Stability of Complete Blood Count and 3-part white cell differential parameters with storage time and temperature variation using Cell Dyn 1800 Automated Hematology Analyzer

BY

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A Research thesis submitted to the Department of Medical Laboratory Sciences, School of Medicine, Addis Ababa University, in partial fulfillment of Master Of Science Degree in Clinical Laboratory Sciences (Specialty Hematology-Immunohematology)

July, 2011

Addis Ababa, Ethiopia

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DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for this thesis has been duly acknowledged.

Name: _____

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Place: Addis Ababa, Ethiopia

Date of submission: July 2011

This thesis has been submitted for examination with my approval as a university advisor.

Name: _____ Signature: _____

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Acronyms

ART- antiretroviral therapy

CBC- Complete blood count

MCV- Mean corpuscular volume

MCH- Mean corpuscular hemoglobin

MCHC- Mean corpuscular hemoglobin concentration

WBC- white blood cells

RBC – Red blood cells

Hb – hemoglobin

Hct- hematocrit

Fl- femto litre

µg- Microgram

Pg-pico gram

RDW- Red cell distribution width

EDTA- Ethyldiaminetetraaceticacid

Abstract

Background: Complete blood count (CBC) and differential white cell counts are the most commonly ordered tests in clinical practice. The standard operation procedures in the ART laboratories, which are prepared based on the instrument instruction manual of the company, recommend 8 hours storage at room temperature. However, there is no clear information as to how to handle delayed specimens in situations where delayance is unavoidable.

Objective: To evaluate whole blood stability with time, storage temperature and transportation conditions using Cell-Dyn 1800, a 3-part diff hematological analyzer.

Methods: A total of 45 adult participants, 19 from ART clinic of Tikur Anbessa Specialized Hospital and 26 apparently healthy Medical Laboratory Science students of Addis Ababa University were included in the study using convenient sampling methods. EDTA whole blood samples were analyzed at baseline before and after aliquoting. Each of six aliquots, for room temperature and 2-8^oC storage, one each for transporting with and without an ice box were used. After the baseline analysis of the direct EDTA tube and an aliquot, all specimens were analyzed at 8, 24, 32, 56 and 72 hours of storage. Same EDTA tube was used each time after refrigeration. Transported samples were analyzed within 8 hours and compared with baseline values. Cell-Dyn 1800 automated hematology analyzer was used following the manufacturers instruction.

Results: Among the CBC parameters, WBC, RBC, Hgb, MCH were stable for 72 hours post sample collection regardless of storage temperature. These values were also unaffected during transportation. MCHC was relatively less stable. PLT counts remained stable in the direct EDTA tube samples stored at 2-8^oC for 72 hours while aliquoted samples were less stable, showing a declining trend with time. The MCV and RDW, parameters affected by change in the red cell size showed an increasing trend with time where differences were statistically significant ($P < 0.05$) after 48 hours of storage. WBC Differential data was the least stable of all, even after 8 hours of storage, where granulocytes showed a declining trend

Conclusion: The various CBC parameters showed different levels of stability at different specimen storage and handling conditions. Each parameter should be treated case by case in conditions where delayance is unavoidable.

1. Introduction

Normal body function and regularly maintained human body physiological make up mainly depends on blood and its constituents. The different types of blood components such as red cells and its main constituent hemoglobin, white cells, platelets, plasma and plasma soluble substances, etc exist only in a certain limited quantities and qualities. Compositions beyond those normal limits are indicative of pathological conditions. It is a common practice in a clinical laboratory to analyze blood samples to make certain that these, especially the cellular components, are within their physiological ranges. Since the different cellular features are the basis for the determination of their quantity, quality and/or functions, briefly describing the natural physiological characteristics of each cell type is critically important to identify and categorize the pathological conditions from normal status (1,2,3,4,5).

Erythrocytes:- Normal red blood cells are biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometer at the thickest part and 1 micrometer or less in the center. They are devoid of most of the cellular organelles especially in its matured stage and yet survive about 120 days of its life span. Some times they are referred to as a bag of hemoglobin since hemoglobin forms about 95% of the intracellular protein of red cells. Nucleus, mitochondria, lysosomes, and golgi apparatus are among the most important components which matured erythrocytes lack. Due to the absence of these vital structures, matured red cells can not synthesize proteins and nucleotides which are relevant to its life existence though it has a limited capability to metabolize some of the molecules such as carbohydrates. Energy extracted from glycolysis is used to maintain the matured red cell and its hemoglobin (1,2,6).

Leukocytes:- The five types of leuokocytes generally categorized in to two major groups mainly based on the availability of granules. Neutrophils (polymorphonuclear cells), Eosinophils and Basophils are granulocytes whereas Monocytes and Lymphocytes are non-granulocytes though in strictly speaking, there are probably no white cells totally

devoid of granules. Granularity and nuclear lobularity including their size difference are major characteristics which automated analyzers use to enumerate and differentiate the different granulocytes. The non-granulocytes are counted based on their size difference and absence of complexity (7). In general, the size of the leukocytes range from 7 – 20 micrometers in which small lymphocytes are the smallest and monocytes are the largest (2).

Platelets:- Platelets or thrombocytes are minute cells 1 to 4 micrometers in diameter. They are cellular fragments derived from the bone marrow megakaryocytes. The megakaryocytes are the largest cells in the hematopoietic cell series where as its immediate successor, the thrombocytes, are the smallest blood cells. Even though platelets lack most important cellular organelles such as the nucleus, they have many functional characteristics among which controlling hemorrhage is the most important (1,2)

Blood cell based laboratory diagnosis is done for several reasons. Some of the objectives are to determine whether the cell quantity is in the normal limit, to ascertain if the morphology and the composition of cell constituents are under normal physiologic conditions, to see if inclusion bodies such as parasites are available in the cell, to observe whether there is any sort of malignant cellular growth. Quantitative and/or qualitative blood cell testing is regularly performed since changes have a critical clinical significance on the health status of the individuals (5,7).

Complete blood count (CBC) and differential white cell count are the most commonly ordered tests to confirm these blood conditions. A complete blood count is a series of tests used to evaluate the composition and concentration of the cells and cellular components of blood. At least nine blood cell parameters are categorized under CBC. Evaluation of these components offers a variety of hematological information to interpret and review that directly relate to the health of the blood producing organ (bone marrow) which is represented by the numbers and types of cells in the peripheral circulation. The nine

components of the CBC are the white blood cell (WBC), red blood cells (RBC), hemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular hemoglobin concentration or content (MCHC), platelet count, and red cell distribution width (7,8). The five white cell differential count also includes the three granulocytes and the two non-granulocyte families.

Measurements of blood cells whether it is quantitative or qualitative, is done both manually and using automated techniques. Though a manual method in resource poor countries is still dominant, automation is also getting great emphasis and rapidly expanding for its accuracy, precision, and speed. Automated hematology analyzers are able to enumerate and/or determine the various hematological parameters in order to screen the presence and types of different abnormalities(5,7). Depending on the type of automated machines used, some of the blood cells parameters are directly read from the instrument and other values are derived by calculation. WBC, RBC, platelet counts, Hb and MCV are directly read while Hct, MCH and MCHC are calculated using RBC and hemoglobin data.

A variety of automated instruments are now commercially available. These machines determine the differential white blood cell count giving either the five or three part differential and in some case the reticulocytes in EDTA whole blood. Depending on their type, these instruments can also analyze cells from body fluids and bone marrow. Although there are various types of measurement technologies employed in hematological determinations, the two most popular are Impedance measurement and optical or light scatter methods (5, 9,10).

Blood is one of the most perishable substances especially when kept *in vitro*. During storage, the amount of blood cells and their morphology may change as a result of various processes such as the effects of glass or plastic tube adsorption characteristics, protein denaturations, evaporations of some volatile constituents, in and out flow of water resulting in hemoconcentration, and continuing metabolic activities of leukocytes and

erythrocytes. These changes occur at a different rate in blood samples stored at room temperature or refrigerated. Therefore, storage requirements of the different blood constituents vary widely (11).

Based on the above described facts, laboratory results are often influenced by the time between blood collection and measurement, as well as storage conditions. Since the constituents deteriorate over time, test analysis should be done within a limited period of time to achieve accurate and reliable results. Stability time is described by different automated hematology analyzer manufacturers though the data varies from company to company (12). In addition, some studies provide relevant information that requires further confirmations to accept specimens older than 24 hours for CBC parameters (13). As a result, this study is designed to determine the storage stability time of blood cells kept at room and refrigerator (2⁰C to 8⁰C) temperatures.

1.1. Statement of the problem

All types of hematological determinations by the laboratory method can be accurately and appropriately done by considering all normal cellular features. Cellular elements are known to have limited stability in ethylenediaminetetraacetic acid (EDTA) anticoagulated blood. Furthermore, the different behaviors of automated counters using either impedance or optical methods may have an effect on delayed samples which should be taken into considerations (14). Deviance of blood cells forms, shapes and/or compositions from the natural features affects the laboratory test result and the difference can be brought due to various reasons including external factors.

Test delayance is one of the major external factors which causes loss of stability of cellular features which in turn leading to wrong test results. For example, technologies most commonly rely upon cell size or cell size together with scatter properties to differentiate white blood cell populations leads to unreliable results if analysis done on delayed samples (15). Stability has been defined by International Standard Organization (ISO) as the

capability of a sample material to retain the initial property of measured constituent for a period of time within specified limits when the sample is stored under defined conditions (ISO guide 30, 1992) (16). Instability is present when there are important changes in one or more of those measurements. The time interval between collection and testing including sample transport and storage time can have an important effect on the quality of test results.

Blood specimens are frequently delivered to the clinical laboratory after a significant post-collection interval. On weekends, this interval may exceed 72 hours, at which time period the reliability of the test result is compromised and clinical significance is violated. So many factors can be mentioned for the late arrival of samples among which a referral based testing is the most prominent. Ethiopian healthcare delivery is organized on a tier system which applies a referral networking method of laboratory services where the smaller laboratories at the lower health facility send specimen to the next higher laboratory for different reasons (17). The average distance samples referred are 53kms according to the information from unpublished material of the Ethiopian Health and Nutrition Research Institute (18). These samples are transferred for automations for one or more of the following reasons; in case of the absence of the machine in a referring laboratory, for confirmatory reasons or retesting, whenever there are machine breakages, power interruptions, lack or absence of the required professional or manpower, etc.

In Ethiopia, with the scale up of free ART access in several health facilities with a specimen referral system to testing laboratories equipped with automated facilities, it is not uncommon for testing laboratories to receive samples older than the 8 hours time recommended by one of the manufacturers of the widely distributed analyzer called Cell Dyn 1800 (12). Cell Dyn 1800 is a hematology analyzer designed to accept a di-potassium EDTA anticoagulated blood specimen. It performs CBC and the three part white cell differential using electrical impedance principle of cell enumeration and modified methemoglobin method for hemoglobin photometry (12). Impedance is defined by this

machine's manual as 'a process that detects and sizes cells suspended in a conductive medium as they are drawn through an aperture so that each cell creates a resistance to current flow that is directly proportional to its own volume. This electrical impedance is used to count the white blood cells, red cells and platelets as they pass through an aperture and producing pulses. The number of pulses during a cycle corresponds to the number of cells counted. The amplitude of each pulse is directly proportional to the cell volume. This machine also uses electronic sizing to determine a three part automated differential. The percentage and absolute counts are determined for lymphocytes, granulocytes (mainly neutrophils), and mid size population of monocytes, basophils eosinophils, blasts, and other immature cells (12). Some parameters like that of hematocrit, MCH and MCHC are also calculated from the data of red cell count and hemoglobin by this instrument. The critical question then is how stable the hematological cells and their components are when kept under different conditions for a different time period (13).

A baseline data or a certain reference must be in place to decide acceptance or rejection of such samples by the laboratory staff. In addition, if specimen is accepted, whether to perform all of the ordered tests or only those deemed appropriate based on the age of the specimen and what comments, if any, should be appended to the reported results regarding their reliability or unreliability. Such decision making requires laboratorians to be familiar with changes known to occur in blood specimen during storage (13,14).

1.2. Literature review

Manufacturers of automated analyzers and some literatures often indicate that blood specimens kept at either room temperature or refrigerated at 2⁰C to 8⁰C can be stable for certain hours and generally give reliable results for some hematological parameters but not for the others. However, specific information concerning the suitability or unsuitability of specimens for the different laboratory tests is scarce (13,19). Manufacturers recommend each laboratory establish its own standards of acceptability for results on samples held beyond the optimal time for analysis (15). As previously stated, the problem of deciding

suitability or unsuitability is especially more critical in areas where testing is done on a referral basis.

To determine sample stability or aging time, different studies have been conducted using different analyzers. From the various analyzer types, this review focused on certain selected instruments which are and which will be relevant for the country.

Cell Dyn is one of the commonly known hematology analyzer. Specific models reviewed for this study include: Cell Dyn 1700, Cell Dyn Sapphire, Cell Dyn 3200, and Cell Dyn 4000. The stability study of WBC, RBC and platelet was studied at room temperature and at 4⁰C for 72 hours using Cell Dyn 3200. WBC count showed a significant decrease after 15 hour storage at both temperatures. Significant differences here also noticed after 15 hours in neutrophils and lymphocytes but eosinophils, monocytes and basophils were changed after 12, 18 and 24 hours, respectively, at room temperature. Leukocyte differential result was not changed for 72 hours stored at 4⁰C. Based on this evaluation, 4⁰C storage is recommended since only the WBC count was changed after 15 hours (20)

Another study evaluated the aging of complete blood count and leukocyte differential parameters using Cell Dyn Sapphire and demonstrated no substantial change in CBC parameters in the range of 24 – 48 hours at room temperature except in the optical method of platelet counting. Impedance platelet count is indicated as a better method here if the samples' age is greater than 24 hours. Differential count with the exception of eosinophils was stable for up to 48 hours at room temperature. CBC was stable for 72 hours at 4⁰C except mean platelet volume which showed a slight increase between 48 and 72 hours (14).

In the evaluation of cell Dyn 1700 CS, (which determines 18 parameters including three part differential), samples were stable for RBC and other RBC based parameters such as red cell indices and hematocrit for 12 hours. Results were different for different

parameters after 12 hours. Accordingly, a slight increase in MCV, a fall in RBC count and hematocrit were observed in a 48 hour period (21).

Platelets showed a gradual loss starting from the 6th hour and goes up to 48 hours. On the other hand WBC was constant for 24 hours and there was a slight increase after 48 hours. Percentages of neutrophils and lymphocytes were stable for 8 hours, then a noticeable decrease in neutrophil and increase in lymphocyte observed. In this evaluation, samples with leukocytosis or leucopenia showed a similar time of stability as those with leukocyte count within the normal range (21).

White blood cell count was decreased significantly between 24 and 48 hours particularly in samples stored at room temperature when measured by Cell Dyn 4000. A change in white cell differential was not significant in samples stored for 48 hours at room temperature with the exceptions of a minor reduction in an eosinophil count (22).

Taken together, studies done using the different models of Cell Dyn demonstrated that, CBC is stable from 12 to 24 hours when stored at room temperature and up to 48 to 72 hours at a refrigerator temperature (14,21,22). Cell Dyn 3200 had a different result from others. In this model, WBC count showed a difference starting from 15 hour storage even at 4⁰C. White cell differential counts were within a reasonable stability up to 48 hours both at room temperature and at 4⁰C except eosinophils that showed a minor reduction at room temperature (20)

Evaluations using the different Coulter models (Gen-S, AcT compact 5diff, AcT 5diff cap pierce) have been done from samples stored at room temperature only. AcT 5diff cup pierce Coulter was evaluated in Ontario, Canada (23). In the evaluation, samples from four patients were analyzed over a 54 hour period at room temperature. Accordingly, the CBC parameters were very stable for the 54 hour period. MCV increased over the 54 hours with

a slight increase in RDW (23). A 72 hours follow up study was also conducted using Beckman Coulter compact 5-part differential hematology analyzer. Five patient samples were analyzed in the stability study at selected intervals within the 72 hours. As a result, CBC parameters were very stable and white cell differential showed acceptable stability over the 72 hours time. The differential parameters showed acceptable stability. The values of neutrophils and lymphocytes were stable till 48 hours though there was a minor shift in both cells 9 hours after sampling but with no clinical significance. The monocytes increased slightly after 24 hours (24). On the other hand, based on the evaluation done by Vogelaar using Beckman Coulter of unmentioned model, the variables of white blood cells, platelets, and platelet volume could be done up to 48 hours if stored at room temperature. However for white cell differential, fresh analysis or refrigeration is recommended (25)

Tsuda and colleagues from Japan measured the performance of the Sysmex-2100. One of the parameters to be measured was the sample stability in which CBC was stable for up to 8 hours of storage regardless of temperature. Platelet count was decreased after 24 hours at room temperature. On the contrary, MCV increased within this time range at room temperature but not at 4⁰C. At room temperature, the changes in percentages were, 10% for neutrophils, lymphocytes, and monocytes stored up to 48 hours. The change in basophil appeared higher. Changes in the refrigerated specimen were different from that of room temperature which were indicated within 3% range for neutrophils, and lymphocytes (26). Other stability studies conducted at both room temperature and 4⁰C showed no significant variation for WBCs, Platelet, Hgb, and RBCs during the 68 hours but changes due to cell size caused a change in MCV, Hct, and RDW in this time frame when tested also by Sysmex XE-2100. These parameters are stable for 36 hours at 4⁰C. Samples stored at room temperature began to show RBC size change at 12 hours and by 24 hour the change became significant. Automated differential parameters showed acceptable stability at room temperature and 4⁰C during the 68 hour test (27). A similar study was also done by Baca (Baca et al, 2006) using this automated analyzer. Reliable results were obtained for WBC,

RBC, HgB, and platelet counts from specimens up to 3 days old. But some diff parameters such as neutrophils were not reliable from a day or older specimens (28).

Other studies using Adiva (Bayer healthcare, Diagnostics division, Tarrytown, NY, USA) demonstrated that blood specimens stored for as long as 24 hours at 4⁰C may be suitable for hematologic testing (29). Similarly, the CBC results obtained from Celltack F, MEK-8222 (Nihon Kohden, Tokyo, Japan) was comparable to Cell Dyn 4000 and white cell differentials were acceptable for 24 hours (30).

1.3. Significance of the study

In summary, most laboratories in the health sector of Ethiopia, which especially are engaged in Anti-Retroviral Therapy (ART) provision in the public health facilities, commonly use limited types of automatic hematology analyzers though numerous models are available in the market. The most common instruments are Cell Dyn (Abbott Diagnostics, Abbott Park, IL), Sysmex (Sysmex, Kobe, Japan) and Coulter (Beckman Coulter, Miami, FL). Of the three kinds, Cell Dyn 1800 is the most dominantly distributed and used analyzer in ART laboratories all over Ethiopia.

The standard operation procedures in the ART laboratories prepared based on the Manufacturer's instruction manual recommend 8 hours storage at room temperature and there is no clear information as to how to handle delayed specimens especially those transported from referring laboratories. This study, therefore, aims to determine changes that occur in various parameters of automated hematological tests during storage of blood at room temperature and refrigerator for at least three days using Cell Dyn 1800. The result obtained will indicate the proper stability time and storage temperature for each CBC and differential parameters so that laboratories will have a defined stability time to either accept or reject incoming samples accordingly.

2. Objectives of the study

2.1. General objective

To evaluate whole blood stability with time and storage temperature using Cell-Dyn 1800, a 3-part diff hematological analyzer.

2.2. Specific objectives

- To determine the effect of time on stability of whole blood for CBC and white cell differential counts
- To determine the effect of storage temperature for CBC and leukocyte differential determination.
- To evaluate the effects of sample transportation with and without cold box storage for determinations of CBC and white cell differential

Hypothesis

Storage time more than 8 hours, as recommended by the manufacture of Cell-Dyn 1800 hematological analyzer, and storage temperature affects CBC and white cell Differential counts.

3. Materials and methods

3.1. Study design:

Laboratory testing to determine the number and constituents of human blood cells was done as a cross-sectional study for three days (72 hours). Measurement was carried out to evaluate the effect of post-draw storage time, temperature and transportation on CBC and differential white cell count parameters using Cell-Dyn 1800, a 3-part Diff analyzer.

3.2. Study site

The study was carried out at the Department of Medical Laboratory Science and Tikur Anbessa Specialized Hospital of the College of Health Sciences, Addis Ababa University.

3.3. Study period:

Participant recruitment, sample collection and laboratory testing was done between December 15/ 2010 and January 14/2011.

3.4. Population:

3.4.1. Source population: All patients attending Tikur Anbessa Specialized Hospital of Addis Ababa University and apparently healthy health science students of Department of Medical laboratory Science.

3.4.2. Study population: All ART patients referred to hematology laboratory of Tikur Anbessa Specialized Hospital of Addis Ababa University and apparently healthy health science students.

3.4.3. Sampling procedures and sample size: Using a convenient sampling procedure, whole blood from 45 individuals was collected using EDTA containing vacutainer tube. Nineteen ART attendants from Tikur Anbessa Specialized Hospital and 26 year two and year three students from school of medical laboratory sciences participated in this study. Patients on ART follow up were recruited according to their arrival to the hospital laboratory and all volunteer students were taken considering their age and sex.

3.4.4 Inclusion and exclusion criteria: all participants were adults aged greater than 18 years. In addition, there were no pregnant women.

3.5. Study variables

3.5.1. Dependent Variable: CBC and differential count data

3.5.2. Independent Variable: storage time, storage temperature, transport condition

3.6. Measurement and Data collection

3.6.1. Specimen collection, testing and storage- From each study participant, about 8 to 10 milliliter whole blood was collected in an Ethylene diaminetetraacetic acid (K₂EDTA) vacutainer tube. The participants were 19 ART patients and 26 students all aged greater than 19. Baseline test analysis was performed for each whole blood before aliquoting and then, using a micropipette, each individual sample was aliquoted into 15 cryovials about 400 to 500 microlitre each with gentle mixing between every aliquot. Baseline testing was also done for one of the aliquoted samples. Then the left over samples in the EDTA vacutainer tube were stored in a refrigerator for parallel testing starting the eighth hour. For each study participant, out of the 15 cryovials, one tested at baseline, six cryovials were kept at room temp while the other group of six were stored at 2-8⁰C for testing at the different time points (8, 24, 32, 48, 56 and 72 hours). The remaining two cryovials were used to evaluate the effect of transportation and handling during transportation by keeping one in an ice box containing ice packs and the other one in the open air in the vehicle. Both sets of samples with and without ice box were transported about fifty kilometers (thirty km rough gravel and 20 kms asphalt road) by a vehicle. All the transported samples were analyzed within eight hours after collection.

3.6.2. Laboratory Testing- laboratory analysis was performed using Cell Dyn 1800 hematology analyzer (*Abbot Diagnostics, Abbot park IL, USA*) in Tikur Anbessa hospital at the following time intervals. Baseline, 8 hours, 24 hours, 32 hours, 48 hours, 56 hours and 72 hours. Each test was done from a newly opened cryovial stored at room and refrigerator temperatures. In addition, parallel testing from left over samples was also done from the original vacutainer tube. Here repeated opening and closing was mandatory and few of the samples were not tested in all the specified times because of inadequacy. Two vials from each sample group were also analyzed to measure transportation effects. Blood cell parameters assayed were WBC, RBC, PLT, Hb, Hct, MCV, MCH, MCHC, RDW, PDW, differential counts of Lymphocytes,

Granulocytes and MID cells. The instrument used electrical impedance technology in counting and sizing.

3.6.3. Quality Control- sample collection and aliquot preparation was done by highly experienced laboratory staffs. Test analysis was done by one of the highly experienced laboratory technologist in Tikur Anbessa and by the student who was doing the study (senior technologist). The Cell Dyn 1800 analyzer's with-in run precision was measured using ten replicate testing's from a single specimen. In addition, the instrument was quality controlled with low, normal and high commercial controls (*Abbot Diagnostics, Abbot park IL, USA*) at least once a day. Room and fridge temperature were measured twice a day, morning (10AM) and after noon (2PM). Range of temperature variation was 19⁰C to 24⁰C for room temperatures and 2⁰C to 6⁰C for refrigerator temperature. The ambient room temperature is 18⁰C to 25⁰C while expected refrigerator temperature to store whole blood is 2⁰C to 8⁰C.

3.7. Data Analysis

Data was entered into excel and analyzed using STATA version 10 statistical package (STATA corporation, College Station Texas, United States). Precision of the instrument was determined by analyzing ten replicates of a single sample and calculating the coefficient of variation. The CV obtained was compared against the CV provided by the instrument manufacturer. In the evaluation of sample stability at the different time points and storage conditions, the means of the results found at the eighth, twenty fourth, thirty two, forty eighth, fifty sixth and seventy two hours post collection were calculated. All the means were compared against the baseline mean values using the student's paired t-test. P values <0.05 were considered as statistically significant.

3.8. Ethical Considerations

Ethical clearance and procedures for informed consent was obtained during the approval of the proposal by the Institute of Review board (IRB) in the Medical faculty, Addis Ababa University. Accordingly, the significance of the study was explained to the study

participants and agreement was reached. Coded samples were used for the analysis. ART participants were on their routine follow up visit and hematological investigations were part of the tests performed at each visit. Confidentiality was maintained throughout the study period.

4. Result

4.1. Precision studies

A total of 45 EDTA whole blood samples were collected from 19 adult HIV positive patients and 26 apparently healthy volunteer Medical Laboratory Science students. Since the study aimed at investigating the effect of storage and timing on sample stability, precision of the analyzer was first evaluated for the different parameters included in the study. To this effect, 10 replicates of the same sample were analyzed to determine within run precision using the percent coefficient of variation. Table 1 summarizes precision results as compared to values provided by the manufacturer of the Cell-Dyn 1800 automated hematological analyzer. Since the manufacturer provided data only for five parameters (WBC, RBC, Hgb, MCV and PLT), comparison was restricted to these parameters. As shown in the table, precision values were below the cutoff given by the manufacturer indicating that any difference obtained in the current study are not attributable to analytical variations.

4.2. Comparison of baseline values by tube types

Since the study involves the analysis of samples directly from the EDTA vacutainer tube and after taking aliquots, mean baseline values obtained from EDTA tube and aliquoted samples were compared for all the parameters included in this study. As shown in Table 2, there was no statistically significant difference ($P>0.05$) between CBC parameters generated from direct EDTA tube and aliquots.

Table 1. Precision studies to determine within run variations using Cell-Dyn 1800

No	WBC (x 10 ⁹ /L)	LYM (%)	MID (%)	Gran (%)	RBC (x 10 ¹² /L)	Hgb (gm/dL)	Hct (%)	MCV (fL)	MC H (pg)	MCH C (%)	RD W	Plt (x 10 ⁹ /L)
1	4.4	18.8	13.7	67.4	5.05	16.0	47.0	93.1	31.4	34.0	13.9	219
2	4.7	21.0	15.5	63.5	5.09	16.3	47.0	92.3	32.0	34.7	13.5	227
3	4.5	18.4	14.1	67.5	5.22	16.3	48.1	92.1	31.2	33.9	13.4	230
4	4.5	19.0	14.5	66.5	5.12	16.2	47.0	91.8	31.6	34.5	13.6	219
5	4.6	19.7	14.4	65.9	5.15	16.2	47.7	92.6	31.5	34.9	14.0	226
6	4.5	20.4	13.0	66.6	5.03	16.0	46.2	91.9	31.8	34.6	13.8	215
7	4.6	20.5	13.4	66.1	5.06	16.2	46.3	91.6	32.0	35.0	13.5	218
8	4.7	21.8	13.4	64.8	5.05	16.3	46.3	91.7	32.3	35.2	14.5	219
9	4.5	23.6	12.6	63.8	5.14	16.4	47.3	92.0	31.9	34.7	13.4	231
10	4.7	23.3	11.9	64.8	5.12	16.3	46.0	91.5	31.8	34.8	14	225
Mean	4.57	20.7	13.7	65.7	5.10	16.2	46.9	92.06	31.8	34.5	13.8	223
S.D	0.1	1.8	1.0	1.4	0.1	0.1	0.7	0.5	0.3	0.4	0.4	5.6
%CV	2.3	8.7	7.6	2.1	1.1	0.8	1.5	0.5	1.0	1.3	2.5	2.5
CPD*	≤ 2.5				≤ 1.7	≤ 1.2		≤ 1.5				≤ 6

*CPD- company's precision data (C.V)

Table 2. Comparison of baseline CBC values generated from direct EDTA tube and Aliquot (N=45)

Parameters	EDTA Tube baseline	Aliquote baseline	P-value
WBC (x 10 ⁹ /L)	6.93 [2.0]	6.77[2.0]	0.7043
LYM (%)	39.53[9.8]	38.86[9.8]	0.7508
MID (%)	9.06[2.3]	9.07[2.1]	0.9850
Gran (%)	51.39[10.1]	52.14[10.8]	0.7436
RBC (x 10 ⁹ /L)	4.96[0.72]	5.01[0.70]	0.7551
Hgb (gm/dL)	14.97[1.6]	15.12[1,6]	0.6737
Hct (%)	42.06[4.2]	42.08[4.2]	0.9842
MCV (fL)	85.56[7.9]	84.07[10.3]	0.4497
MCH (pg)	30.46[3.0]	30.48[3.0]	0.9721
MCHC (%)	35.58[0.7]	35.87[0.7]	0.0531
RDW	14.56[1.5]	14.59[1.6]	0.9254
Plt (x 10 ⁹ /L)	253.96[124.0]	237.93[120.1]	0.5409

4.3. Effects of time and temperature variation on WBC count

After confirming the precision of the instrument (Table 1), all other results from the different time points under different storage conditions were compared with the baseline data. That is, EDTA tube results from 8, 24, 32, 48, 56, and 72 hours were compared with the baseline EDTA tube results. On the other hand, all the results obtained from a cryovial container (aliquots) during the same time points with different sample storage, handling and transportation conditions were compared from cryovial (aliquoted) baseline results.

Table 3 summarizes mean \pm SD data and per cent changes from the baseline values under different storage and handling conditions at different time points for WBC counts. Accordingly, the data revealed that WBC counts remain almost stable up to 72 hours for counts performed from the original EDTA tubes (stored refrigerated after each analysis). The same stability pattern was observed for aliquoted but refrigerated cryovial tubes while aliquoted samples stored at room temperature showed a slightly increasing pattern as indicated by the percent change (Table 3). However, under all conditions the difference was not statistically significant ($P>0.05$).

Comparison between the different tubes (EDTA refrigerated, Aliquoted room temp and aliquoted refrigerated) showed no statistically significant difference at all time points except for aliquoted room temperature and refrigerated EDTA tube results, which was marginally significant at 72 hours. The results from aliquoted and room temperature stored samples were higher compared to the values obtained from EDTA refrigerated tubes ($P=0.0492$).

Table 3. Effects of time and temperature variation on WBC count (x 10⁹/L). Data are presented as mean ± SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	6.9 ± 2.0 (100)	6.8 ± 2.0(100)	6.8± 2.0(100)
8 hr	6.9 ± 1.6 (100)	6.9 ± 2.1 (101.8)	7.0± 2.1(102.9)
24hr	7.2 ± 2.1(104.3)	7.2± 2.2 (105.9)	6.9± 2.1(101.5)
32hr	7.0 ± 2.1(101.4)	7.1± 2.1(104.4)	6.9± 2.1 (101.5)
48hr	7.0 ± 2.0(101.4)	7.3 ± 2.3 (107.4)	6.9± 2.0(101.5)
56hr	6.8 ± 1.9 (98.6)	7.3 ± 1.9 (107.4)	6.9± 2.1(101.5)
72hr	6.5 ± 1.9 (94.2)	7.4 ± 1.9(108.8)	6.9± 2.2(101.5)

4.4. Effects of time and temperature variation on Lymphocyte count

The three part differential results which include Lymphocytes, Mid range cells (mainly monocytes) and granulocytes was also evaluated against the baseline results. Accordingly, results of lymphocytes found from EDTA tube samples increased significantly after 56 hours at $P \leq 0.05$ when compared with the baseline data. Moreover, the percent lymphocyte values obtained from the EDTA tube were significantly higher compared to room temperature stored aliquoted vials at 24 hours ($P=0.03$), 32 hours ($P=0.08$; marginally significant), and 48 hours ($P=0.04$). As shown in Table 4, under all conditions, proportion of lymphocytes showed an increasing trend with time although the result reached a statistically significant level for EDTA tubes at 56 and 72 hours ($P < 0.05$).

4.5. Effects of time and temperature variation on MID range cells

MID range cells results were not significantly varied up to the 24th hour in EDTA tube samples kept refrigerated and up to 8 hours in aliquoted samples. These groups of cells which are mainly composed of the monocytes showed an increasing trend with time regardless of storage condition. Some inconsistencies with storage time were noted in aliquoted refrigerated samples. The magnitude of the changes were more exaggerated in samples stored at room temperature that showed a statistically significant ($P < 0.05$) value even at the 8th hour and continued increasing consistently till the end of the three days evaluation period (Table 5). A percent change of even more than 200% was noted at the end of the evaluation period.

Table 4. Effects of time and temperature variation on Percent Lymphocyte count. Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	39.5 \pm 9.8(100)	38.9 \pm 9.8(100)	38.9 \pm 9.8(100)
8 hr	38.7 \pm 11.1(98)	39 \pm 9.7(100.3)	39.4 \pm 9.9(101.3)
24hr	40.6 \pm 9.9(102.8)	35.9 \pm 9.8 (92.3)@	39.4 \pm 10.0(101.3)
32hr	41.6 \pm 9.9(105.3)	38.0 \pm 9.5(97.7)@	40 \pm 9.5(102.8)
48hr	42.4 \pm 10.2(107.3)	38.1 \pm 9.4 (97.9)@	40.3 \pm 9.8(103.6)
56hr	43.4 \pm 9.9(109.9)*	41.8 \pm 9.2(107.5)	42.5 \pm 9.4(109.3)
72hr	43.6 \pm 10.1(110.4)*	40.6 \pm 9.2(104.4)	42.6 \pm 9.3 (109.5)

* Significant difference compared to baseline (P<0.05)

@Significant difference compared to EDTA tube of same time point (P<0.05)

Table 5. Effects of time and temperature variation on percent MID cell counts. Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	9.1 \pm 2.3 (100)	9.1 \pm 2.1(100)	9.1 \pm 2.1 (100)
8 hr	9.8 \pm 2.5(107.7)	10.8 \pm 2.2(118.7)*	10.8 \pm 2.5(118.7)*
24hr	9.5 \pm 4.2 (104.4)	15.6 \pm 7.3 (171.4)*	8.8 \pm 2.0 (96.7)*
32hr	12.9 \pm 4.5(141.8) *	18.8 \pm 4.1(206.6) *	9.8 \pm 2.7(107.7) *
48hr	15.1 \pm 5.4(165.9) *	20.7 \pm 3.7 (227.5) *	11.4 \pm 3.8(125.3) *
56hr	17.0 \pm 5.0(186.8) *	22.5 \pm 3.8 (247.3) *	13.7 \pm 4.7(150.5) *
72hr	17.9 \pm 5.0(196.7) *	22.4 \pm 3.5(246.2) *	14.8 \pm 5.6(162.6) *

*Significant difference compared to the respective baseline counts (P<0.05)

4.6. Effects of time and temperature variation on Granulocytes

As summarized in Table 6, proportions of Granulocytes declined remarkably with time regardless of storage condition and tube types though statistical significance level reached at 32 hours (P<0.05) for EDTA tube and room temperature stored aliquoted sample. On the other hand, difference was marginally significant (P=0.09) at 48 hours for refrigerated aliquoted sample.

Table 6. Effects of time and temperature variation on percent Granulocytes. Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	51.4 \pm 10.1 (100)	52.1 \pm 10.8(100)	52.1 \pm 10.8 (100)
8 hr	51.6 \pm 11.8(100.4)	50.3 \pm 10.8(96.5)	49.8 \pm 11.0 (95.6)
24hr	49.9 \pm 11.5 (97.1)	48.5 \pm 11.2(93.1)	51.6 \pm 10.7 (99.0)
32hr	45.5 \pm 11.5(88.5)*	43.2 \pm 10.5(82.9) *	50.2 \pm 10.2 (96.4)
48hr	42.5 \pm 12.1(82.7) *	41.2 \pm 10.6 (79.1) *	48.3 \pm 10.5 (92.7) *P=0.09
56hr	39.6 \pm 11.4(77.0) *	35.6 \pm 9.9(68.3) *	43.9 \pm 10.9 (84.3) *
72hr	38.5 \pm 11.7(74.9) *	37.1 \pm 9.6(71.2) *	42.6 \pm 11.3 (81.8) *

*Significant difference compared to baseline values (P<0.05)

4.7. Effects of time and temperature variation on RBC count

Table 7 shows mean values and percent changes for red cell counts. As shown in the table, RBC count remained stable regardless of tube type, storage condition and handling throughout the three days evaluation time.

Table 7. Effects of time and temperature variation on Red Blood Cell count ($\times 10^{12}/L$). Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	5.00 \pm 0.72(100)	5.01 \pm 0.70(100)	5.00 \pm 0.70(100)
8 hr	5.02 \pm 0.82(100.4)	4.99 \pm 0.71(99.8)	4.99 \pm 0.71(99.8)
24hr	4.96 \pm 0.69(99.2)	5.03 \pm 0.69 (100.6)	5.00 \pm 0.71 (100)
32hr	4.96 \pm 0.68(99.2)	4.98 \pm 0.69(99.6)	5.00 \pm 0.71(100)
48hr	4.94 \pm 0.70 (98.8)	4.98 \pm 0.71(99.6)	5.07 \pm 0.69(101.4)
56hr	4.91 \pm 0.68(98.2)	4.92 \pm 0.69(98.4)	4.94 \pm 0.70(98.8)
72hr	4.94 \pm 0.72 (98.8)	4.94 \pm 0.71 (98.8)	4.98 \pm 0.70 (99.6)

4.8. Effects of time and temperature variation on Hemoglobin and Hematocrit levels

As shown in Table 8, Hgb values remained stable in all conditions throughout the 72 hours. On the other hand, the hematocrit value increased to a statistically significant level at 56 (P=0.05) and 72 hours (P<0.05) in aliquoted samples stored at room temperature. Table 9 shows the detailed data for Hematocrit.

Table 8. Effects of time and temperature variation on Hemoglobin determination (in gm/dL). Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	15.0 \pm 1.6(100)	15.1 \pm 1.6(100)	15.1 \pm 1.6 (100)
8 hr	14.7 \pm 2.1(98)	15.2 \pm 1.6(100.7)	15.1 \pm 1.6(100)
24hr	15.1 \pm 1.6(100.7)	15.4 \pm 1.6(102)	15.2 \pm 1.6(100.7)
32hr	15.0 \pm 1.6(100)	15.3 \pm 1.6(101.3)	15.2 \pm 1.6(100.7)
48hr	15.0 \pm 1.6(100)	15.3 \pm 1.7 (101.3)	15.5 \pm 1.8 (102.6)
56hr	15.0 \pm 1.6 (100)	15.1 \pm 1.6(100)	15.1 \pm 1.6 (100)
72hr	15.2 \pm (101.3)	15.4 \pm 1.6(101.3)	15.3 \pm 1.6 (101.3)

Table 9. Effects of time and temperature variation on Hematocrit determination (in %). Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	42.1 \pm 4.2(100)	42.1 \pm 4.2(100)	42.1 \pm 4.2 (100)
8 hr	41.4 \pm 5.5 (98.3)	42 \pm 4.2(99.8)	42.0 \pm 4.3 (99.8)
24hr	41.8 \pm 4.1(99.3)	42.5 \pm 4.1 (101)	41.9 \pm 4.3 (99.5)
32hr	42.1 \pm 4.3(100)	42.6 \pm 4.3(101.2)	42.0 \pm 4.3 (99.8)
48hr	42.2 \pm 4.3 (100.2)	43.6 \pm 4.3 (103.6)	42.5 \pm 4.7 (101)
56hr	42.1 \pm 4.3(100)	43.8 \pm 4.1(104)*P=0.05	41.5 \pm 4.1 (101)
72hr	42.8 \pm 4.5 (101.2)	44.7 \pm 4.2(106.2)*	41.8 \pm 4.1 (99.3)

*significant difference compared to baseline values (P<0.05)

4.9. Effects of time and temperature variation on MCV

MCV values follow a similar increasing trend like the hematocrit in room temperature stored samples and the difference was statistically significant at 48 hours and then after ($P < 0.05$). All the other results obtained from refrigerated EDTA tube and refrigerated aliquots were not statistically significantly different from the baseline values ($P > 0.05$). Data are presented in Table 10.

Table 10. Effects of time and temperature variation on MCV values (in femto liter; fL). Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	85.6 \pm 7.9(100)	85.0 \pm 10.3(100)	85.0 \pm 10.3 (100)
8 hr	83 \pm 6.7(97)	84.1 \pm 10.1(98.9)	84.8 \pm 7.7 (99.8)
24hr	85.1 \pm 7.2(99.4)	85.0 \pm 8.0 (100)	84.5 \pm 7.6 (99.4)
32hr	85.6 \pm 7.1(100)	86.5 \pm 8.0(101.9)	84.9 \pm 7.7 (99.9)
48hr	86.1 \pm 7.5(101.2)	88.4 \pm 8.8(104)*	84.5 \pm 7.6 (99.4)
56hr	86.5 \pm 7.3(101.1)	89.9 \pm 8.9(105.8)*	84.8 \pm 7.7 (99.8)
72hr	87.4 \pm 7.8(102.1)	91.4 \pm 9.8(107.5)*	84.7 \pm 7.9 (99.6)

*significant difference compared to baseline values ($P < 0.05$)

4.10. Effects of time and temperature variation on MCH and MCHC

Mean \pm SD values and per cent changes for MCH are summarized in Table 11 and all values were stable throughout the three days evaluation time. In addition, no remarkable change occurred in MCHC values. Table 12 shows the detailed values for MCHC.

Table 11. Effects of time and temperature variation on MCH (in pico gram; pg) testing. Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	30.5 \pm 3.0(100)	30.5 \pm 3.0(100)	30.5 \pm 3.0 (100)
8 hr	29.5 \pm 2.3 (96.7)	30.7 \pm 3.0(100.7)	30.7 \pm 3.2 (100.7)
24hr	30.7 \pm 2.9(100.7)	30.8 \pm 3.0 (101)	30.7 \pm 3.1 (100.7)
32hr	30.5 \pm 2.9(100)	31.0 \pm 3.0(101.6)	30.7 \pm 3.0 (100.7)
48hr	30.7 \pm 2.9(100.7)	31.1 \pm 3.0(102)	30.8 \pm 3.0 (101)
56hr	30.9 \pm 2.9(101.3)	31.0 \pm 3.1(101.6)	31.0 \pm 3.1 (101.6)
72hr	31.0 \pm 3.1(101.6)	31.4 \pm 3.1(103)	31.1 \pm 3.0 (102)

Table 12. Effects of time and temperature variation on MCHC testing (gm/dL). Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	35.6 \pm 0.7(100)	35.9 \pm 0.7(100)	35.9 \pm 0.7 (100)
8 hr	35.3 \pm 0.7(99.2)	36.1 \pm 0.7(100.6)	36.0 \pm 0.7 (100.3)
24hr	36.0 \pm 0.8(101.1)	36.2 \pm 0.7(100.8)	36.3 \pm 0.8 (101.1)
32hr	35.6 \pm 0.8(100)	35.8 \pm 0.7(100.6)	36.1 \pm 0.7 (100.6)
48hr	35.6 \pm 0.7 (100)	35.2 \pm 0.9(98.1)	36.5 \pm 0.7 (101.7)
56hr	35.7 \pm 0.8(100.3)	34.4 \pm 0.8(95.8)	36.5 \pm 0.8 (101.7)
72hr	35.4 \pm 1.0 (99.4)	34.3 \pm 0.9(95.4)	36.7 \pm 0.6 (102.2)

4.11. Effects of time and temperature variation on Red cell distribution width (RDW)

As summarized in Table 13, RDW values did not show a significant difference till 32 hours for aliquoted samples kept at room temperature and original refrigerated EDTA vacutainer tubes. However, the difference from baseline value become statistically significant starting the 48th hour testing time ($P < 0.05$). Nevertheless, refrigerated aliquote sample were found to be stable throughout the three days.

Table 13. Effects of time and temperature variation on RDW estimation. Data are presented as mean \pm SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	14.6 \pm 1.5(100)	14.6 \pm 1.6(100)	14.6 \pm 1.6 (100)
8 hr	15.4 \pm 2.0(105.5)	14.7 \pm 1.5(100.7)	14.6 \pm 1.6 (100)
24hr	14.8 \pm 1.6(101.4)	14.8 \pm 1.8(101.4)	14.5 \pm 1.7 (99.3)
32hr	14.9 \pm 1.5 (102.1)	15.1 \pm 1.6(103.4)	14.7 \pm 1.5 (100.7)
48hr	15.3 \pm 1.7 (104.8)*	15.4 \pm 1.6(105.5)*	14.5 \pm 1.5 (99.3)
56hr	15.3 \pm 1.6(104.8)*	15.6 \pm 1.7(108.6)*	14.6 \pm 1.5 (100)
72hr	15.5 \pm 1.6 (106.2)*	15.4 \pm 1.6(105.5)*	14.3 \pm 1.4 (97.9)

*significant difference compared to baseline values (P<0.05)

4.12. Effects of time and temperature variation on Platelet counts

Platelet results were not reliable after 48 hour and 56 hour in aliquoted samples stored at room temperature and refrigerator, respectively where a decreasing trend was observed in both sample categories. However, EDTA tube results were as good as baseline results until the end of the analysis period. Table 14 demonstrates the platelet data at different times and different storage conditions.

Table 14. Effects of time and temperature variation on Platelet count (x 10⁹/L). Data are presented as mean ± SD, % changes (shown in parenthesis) at different time points. Differences between means were compared using student's *t*-test.

Time	Original EDTA tube	Cryovial aliquoted tube	
		Room Temp.	Refrigerator
Baseline	254.0± 124.0(100)	238.0± 120.0(100)	238.0±120.0 (100)
8 hr	210.1± 124.6(82.7)	211.1± 109.8(88.7)	213.9±106.8 (89.6)
24hr	244.2± 126.9(96.1)	213.2± 115.2(89.6)	209.7±113.9 (88.1)
32hr	245.4±127.1 (96.6)	196.4±103.5 (82.4)	200.8±105.8 (84.4)
48hr	245.4± 127.6(96.6)	189.4± 96.8(79.8)*	211.6±119.9 (88.9)
56hr	239.2± 126.3(94.2)	158.8± 80.1(66.7)*	181.5±105.5 (76.3)*
72hr	246.5±130.7 (97.0)	155.7± 85.1(65.4)*	187.0±103.4 (78.6)*

*Significant difference compared to baseline values (P<0.05)

4.13. Effects of transportation on CBC parameters

To determine the effect of transportation on sample stability, aliquoted samples were transported about 50 kms in Addis with and without ice box storage and analyzed within 8 hours after collection. Results obtained from these two tubes were compared with results obtained from baseline EDTA and aliquoted tubes analyzed at 0 hour. The ice box contains cooling ice packs.

No remarkable difference was noted for all parameters after transportation with either types of modes of transportation, with or without ice box (data not shown).

5. Discussion

The study aimed to demonstrate the effect of storage time, temperature and transportation on hematological values generated using Cell-Dyn 1800, an instrument widely distributed in most ART laboratories in Ethiopia. According to the instrument manual followed by the ART laboratories nationally, blood samples for hematological analysis are recommended to be stored for 8 hours at room temperature. No guideline is available if samples stay longer before analysis for various reasons.

The result reported herein revealed that different parameters of the CBC are affected differently by specimen aging and storage conditions, suggesting no single formula could work best for all parameters. For example, the leukocyte count remained stable throughout the three days evaluation period regardless of storage time and temperature in both aliquoted and original EDTA tube samples. Similar post draw stability was observed for erythrocyte counts, Hgb, and MCH values. The MCH is a value which is obtained from the two stable parameters (RBC and Hgb). This finding is consistent with Hill et al who demonstrated a stable WBC, RBC, Hgb, and MCH values up to 72 hours using Sysmex XT-2000i Hematology analyzer and room temperature stored whole blood samples (15). Similarly, Hedberg and Letho using Cell-Dyn Sapphire hematology analyzer reported the same stability pattern (14). In both studies and our study, MCV values showed an increasing trend indicating the increase in size of erythrocytes with room temperature storage (14,15). The significant increase in hematocrit seen in room temperature stored sample also agrees with these studies (14,15). In our case, the rise reaches to a statistically significant level at 56 hours for room temperature stored aliquoted sample. In automated analyzers, the MCV is directly measured from the height of the impulse generated while a cell passes through a small aperture and the Hct is calculated from RBC and MCV (12).

On the other hand, differential WBC counts were more affected with storage time. In the current study, there was an increasing trend for lymphocytes starting at 56 hours and MID range cells (mainly monocytes) even at 8 hours and a declining trend for granulocytes at 32 hours of testing. Available reports on stability of the differential WBC data with aging of

sample lack consistency. For example, Hill et al reported a stable differential count for up to 48-72 hours with the exception of monocytes, which become progressively lower beyond 24 hours (15). This finding completely contrasts to our finding, which demonstrated a remarkable rise in the MID population that mainly comprises the monocytes (12). In fact, Hill et al noted the difficulty of determining a reason for the observed change (15).

Moreover, while we observed an increasing trend for lymphocytes starting at 56 hours and a declining trend for granulocytes at 32-48 hours, Hedberg and Letho (14) reported a declining trend for both populations when using 4⁰C stored samples. The most likely explanation for the observed results in our study could be, granulocytes (mainly the neutrophils) degranulate with time and fall within the size range of the lymphocytes and monocytes, increasing the latter populations with aging. Monocytes, though morphologically the largest cell in the blood, normally due to the effect of the lytic agent they shrink and fall within a size range smaller than the granulocytes (12). This is because, Cell-Dyn 1800 uses the impedance principle to differentially count cells based on their size (12). Time dependent increase in the monocyte population as a result of granulocyte degranulation during storage has been demonstrated by Bartels and Schoorl who used Sysmex NE-8000 hematology analyzer in their study, substantiating our findings (10).

RDW is a useful parameter in the diagnosis of anemia demonstrating anisocytosis but less commonly utilized in our setting (personal experience). This parameter followed an increasing trend for EDTA tube samples and room temperature stored aliquoted samples. This finding is not consistent with findings elsewhere (15). However, the reason for the stable value observed in aliquoted refrigerated samples in our study is not clear. Since the RDW is a calculated value from the MCV (standard deviation of the mean of the red cell volume divided by MCV) (12,15), a change in this parameter is expected.

Platelet counts in the current study remained stable for up to 72 hours in refrigerated samples while in room temperature stored aliquoted samples, there was a significantly declining trend starting 48 hours post collection. Other studies using the impedance principle have also

demonstrated a stability of platelet counts up to 72 hours regardless of storage temperature (14,15). On the other hand, the optical method of platelet count is reported to give less stable results especially at room temperature stored samples (14,15).

Interestingly, while short post draw stability times were traditionally accepted practices in clinical medicine, among the CBC parameters, Hgb, RBC, and MCH were reported to be stable even up to 7 days, WBC count up to 3 days and PLT counts up to 4 days at room temperature stored samples using automated Beckman coulter counter hematology automated analyzer (13). This study (13) also demonstrated an increase in MCV, HCT, and RDW and a declining trend for MCHC and monocytes with specimen aging. Similar stable pattern after one week storage at room temperature has been reported by Hirase et al (19).

On the other hand, a relatively recent data using Cell-Dyn 3200 demonstrated that RBC, Red cell indices, PLT and WBC differential being stable for 72 hours blood storage at 4⁰C while WBC count decreased after 15 hour storage at 4⁰C (20).

Taken together, data on stability studies revealed varying results depending on the storage temperature (Room temperature versus 2-8⁰C), type of instrument/principle (impedance versus optical) (14,15) and HIV status Hill et al (15). Hill et al demonstrated that blood sample from HIV positive patients are less stable than HIV negatives (15). Despite the progress in laboratory medicine, storage conditions for hematological parameters generally remained less standardized. Thus, care has to be taken when interpreting data analyzed from aged blood samples.

Of note, the Cell-Dyn 1800 is an instrument widely distributed in Ethiopia. The instrument provides 3 part differential data which as demonstrated above showed a declining trend in the granulocyte population after 32 hours of Blood collection. The MID population was unstable even earlier. For a country like Ethiopia where Eosinophilia is a common finding in healthy populations (Kinde et al 2010), reporting the granulocytes merged as one population may not be informative. In addition, the effect of storage time and temperature was not clear as to

which of the granulocytic cells were affected since this cell category is composed of neutrophils, eosinophils and basophils. Manual differential count may be preferable for laboratories having 3 part differential automated analyzers.

Limitations

Though the study has several strengths of being the first of its kind and carried out by senior professional with many years of experience in hematology laboratory, it has its own weakness. Due to small sample size of ART patients, analysis by HIV status was not possible. In addition, to determine the effect of transportation samples were transported in Addis only which does not reflect the effect of transportation in warmer temperatures as in some parts of Ethiopia.

6. Conclusion and Recommendations

6.1. Conclusion

1. Based on the current finding the following CBC parameters remained stable regardless of storage time, temperature and transportation conditions: WBC, RBC, Hgb, MCH. MCHC was relatively less stable
2. PLT counts remained stable in the direct EDTA tube samples stored at 2-8⁰C while aliquoted samples were less stable, signifying that additional steps such as aliquotion may render the most fragile formed elements of the blood susceptible to destruction.
3. MCV and RDW showed an increasing trend with time
4. WBC Differential data was the least stable of all, even after 8 hours of storage, where granulocytes showed a declining

6.2. Recommendations

1. Since the results of the current study as well as reports elsewhere indicated different stability times for the various CBC and differential count parameters, utmost care is needed in the use of these values from aged samples.
2. Results from individual parameters, when needed, can be used separately for the specified period and conditions
3. The medical laboratory science course given for Medical Students (Physicians) should give emphasis on interpretation of CBC and differential parameters, if aged blood is to be used in conditions where fresh blood analysis is impossible

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