Sudan University of Science and Technology

College of Graduate Studies

Department of Biomedical Engineering

Risk Assessment of Auto Hematology Analyzer Operation

تقويم مخاطر التشغيل لجهاز تحليل مكونات الدم الالى

A project submitted in partial fulfillment for the requirements of degree of M.Sc. in bi-omedical engineering

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الآيـــــــــــه

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

قال تعالى :

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

سورة البقرة (32)

Dedication

To my dear

Mother, father,

Sister, brothers,

Teachers,

And my friends for their patience and encouragement. To every person hope to see me successful, with love and respect.

Abeer

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ABSTRACT

Auto hematology analyzer is used widely to measure blood cells which known as complete blood count. Its results medically important for interventions so should be analytically valid. Many factors may affect in the result of auto hematology analyzer which lead to un reliable result like pre analytical handling related to sample conditions and sample stability according to store conditions and the instrument used for measurement may affect on the result due to be affected by some factors . The main objective of this study was risk assessment on auto hematology analyzer operation taking into consideration the three indicators of performance which are accuracy, precision and reliability. A cross sectional study was conducted in 30 medical laboratories in the Khartoum state. Fifteen hospitals, one health center five dispensaries and nine special lab were involved the study. Data was collected using questionnaire in addition to check up and evaluation study of instrument in the day of interview. The main variables in the questionnaire were staff competency (sex, age, qualification and experience), staff and supervisor training, operation, routine maintenance, preventive maintenance, QC, calibration, instrument environment, accuracy, precision and reliability.30 medical laboratory specialists were interviewed and 30 instruments were checked and evaluated. Analysis of data was done by using SPSS version 26 using correlation to measures the association between two variables frequency tables and percent were calculated. The results reveal that some environment and personal elements are signify cant effect on the performance of auto hematology analyzer. appropriate environment effects on HGB precision with coefficient of 0.539(p=0.002), adequate temperature effects on HGB, PLT precision with coefficient 0.484(p=.007),0.583(p=0.001)in series and adequate space effects on PLT , HGB precision with coefficient 0.451(p=0.012) and 0.404(p=0.027).in addition to Standard check effects on WBC precision with coefficient 0.447(p=0.013),preventive maintenance effects on HGB,HCT accuracy with coefficient 0.456(p=0.011),0.393(p=0.032) in series ,QC effects on RBC precision with coefficient 0.363(p=0.049). It conclude that the instrument environment has effect on the precision of HGB and PLT while some personal elements effect on precision of WBC, RBC, accuracy of HGB and HCT.

المستخلص

يستخدم محلل الدم التلقائي على نطاق واسع لقياس خلايا الدم والذى يعرف بتعداد الدم الكامل . نتائج التحليل مهمة فى التداخلات الطبيه لذلك يجب أن تكون صالحة من الناحية التحليلية .قد تؤثر العديد من العوامل في نتيجة محلل الدم التلقائي مما يؤدي إلى نتيجة غير موثوقة بها تتمثل هذة العوامل فى المعالجة التحليلية السابقة المتعلقة بظروف العينة واستقرار العينة وفقًا للظروف التى حفظت فيها العينه وقد تؤثر الأداة المستخدمة للقياس على النتيجة بسبب تأثرها ببعض العوامل .الهدف الرئيسي من هذه الدراسة تقييم مخاطر التشغيل لجهاز تحليل مكونات الدم الالى مع الاخذ فى الاعتبار مؤشرات الأداء الثلاثة وهي الدقة والضبط والموثوقي .أجريت دراسة مقطعية في 30 مختبرا طبيا في منطقة الخرطوم.خمسة عشر مستشفى ومركز صحي واحد وخمسة مستوصفات وتسعة مختبرات خاصة .تم جمع البيانات باستخدام استبيان بالإضافة إلى دراسة فحص وتقييم للأداة في يوم المقابلة .كانت المتغيرات الرئيسية في الاستبيان هي;كفاءةالعاملين (الجنس ، العمر ، المؤهل والخبرة) ، تدريب الموظفين والمشرفين ، التشغيل ، الصيانة الروتينيه، الصيانة الوقائية ، مراقبة الجودة ، المعايرة، بيئة الجهاز ، الصحة، ، الدقة والموثوقية .تمت مقابلة 30 متخصصًا في المختبرات الطبية وتم فحص وتقييم 30 أداة .تم تحليل البيانات باستخدام الإصدار 26 من ال SPSSباستخدام الارتباط لقياس الارتباط بين متغيرين تم تشغيل جداول التكرار وحساب النسبة المئوية.تظهر النتائج أن بعض العناصر البيئية والشخصية لها تأثير كبير على أداء محلل أمراض الدم التلقائي . تأثيرات البيئة المناسبة على دقة HGB بمعامل (P=0.002)0.539 ، وتأثيرات درجة حرارة المناسبة على دقة ال HGB وال PLT بمعامل (P=0.007) 0.484،(P=0.001)0.583 على التوالى وتأثيرات المساحة الكافية على دقة ال PLT وال HGB بمعامل (P=0.012)0.451 و(P=0.027)0.404 بالإضافة إلى تأثيرات الصيانة على دقة ال WBC بمعامل (P=0.013)0.447، تأثيرات الصيانة الوقائية على صحت ال HGB وال HCT بمعامل (P=0.011)0.456 ,(P=0.032) 0.393 على التوالى ، تأثيرات QCعلى دقة RBC بمعامل (P=0.049)0.363. استنتج من ذلك أن بيئة الجهاز لها تأثير على دقة HGB و PLT بينما تؤثر بعض العناصر الشخصية على دقة ال WBC و RBC وصحت ال HGB وHCT .

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LIST OF ABBREVIATIONS

Abbreviation	Definition
µl	Microliter
µm	Micrometer
BFU-E	Burst-Forming Unit Erythoid
C	C: Degree Centigrade
CBC	Complete Blood Count
CE mark:	European Union
CFU-Bs	CFU-Basophil
CFU-Eo	CFU-Eosinophil
CFU-GEMM	Colony Forming Unit Granulocyte-Erythrocyte-Monocyte-Megakaryocyte
CFU-GM	CFU- Granulocyte Macrophage /Monocyte
CFU-MEG	CFU-Megakaryocyte
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
CV	Coefficient of Variation
e.g.	for Example
EDTA	Ethylenediaminetetraacetic Acid
FAD	Food and drug administration
Fl	Femtolitre
FSL	Forward Laser Light
g/L:	gram per liter
Gran	Granulocytes
GSD	Geometric Standard Deviation
h /hr.:	Hour
HA	Hematology analyzer
Hb/Hgb/HGB	Hemoglobin
Hct /HCT	Hematocrit
ICSH	International Council for Standardization in Hematology
ISO	International Organization for Standardization
L	Liter
Lymph	Lymphocyte
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Mid	Mid-Size Cells
Ml	Milliliter
MPV	Mean Platelet Volume
Nm	Nanometer
NRBCs	NRBCs: Nucleated Red Blood Cells
PCT	Procalcitonin

PDW	Platelet Size Distribution
PG	Particle in the Granulocytes Region
Pg	Pico grams
PHSC	Pluripotent Hematopoietic Stem Cell
PL	Particle in the Lymphocyte Region
Plt /PLT	Platelet
PM	Particle in the Mid-Size Region
RBCs	Red blood cells
RDW	Red Cell Distribution Width
RT	Room Temperature
SD	Stander Deviation
SS	Side Scattered
WBCs	White blood cells
Who	World Health Organization

CHAPTER ONE

INTRODUCTION

INTRODUCTION

1.1 General Review

Hematology analyzer (HA) is used widely in patients to count and characterize blood cells, and also to detect and monitor diseases specially anemias, infections, Inflammations, Leukemias, malignancies and Bone marrow failure. (Scoffin K, 2014)

However, the results are medically important for intervention. It is important that the results of hematology analyzer should analytically valid .Therefore, hematology analyzer should be continually assessed to insure that the analytical performance is on international standards and manufacture instructor.

Many studied has been done on HA. In the study of Olga Ciepiela, et al (2016) comprised between three different brands of Automated Hematology Analyzers in all parameters included WBCs, RBCs, and erythrocyte indices as well as platelet count compare it with the manual blood smear.

However, most researches on the HA focus on evaluation or validate the new HA before using in the laboratory to assure that the analytical performance is up to stander or compare the parameter of the new device with the other used .even though the manufacturer validate HA before entre the market in different national and international requirement like FAD (food and drug administration) approve or CE mark (European Union) but this validation is performed under ideal condition. In clinical laboratory practice may differ. (JVIS, HUISMAN, 2016; Noha Bassiouny, et al 2017; Maciel, Tavany et al, 2014).

Therefore there are many parameters may affect the HA practice and analytical result such as environment, user and HA device work condition that may lead to wrong result due to that usually physician doubt about the result but most time return the mistake to the device that used in measurement even if different patients make test in different labs.in addition to absence of medical devices monitoring system to evaluate performance of the devices used in medical institutions.. The main purpose of this study is to find the most effective parameters that may effect on the HA performance.

1.2 Statement of Research Problem

Developing countries suffers from absences of medical instrumentation monitoring system to evaluate performance of the medical instrumentations have already used in medical institutions due to that some patients can face some problem come from medical instrument .There for specifically, this study investigated the risk assessment on auto hematology analyzer operation. The study examined how some parameters affects auto hematology analyzer operation and assessment the extent to which machine environment and some other factors would effect on it.

1.3 Rationale of the Study

1. If a doctor doubts about the medical result then there is mistake in medical instrument used or laboratory process or other reasons.
2. If a medical device gives an unreliable result then there are some factors have affected it.
3. If the medical instrument works for long time then it performance will deteriorate with time

1.4 Objectives

1.4.1 Research Question

What is the effect of some parameters on the auto hematology analyzer result?

Specifically the research aims to answering the following questions:

1. What is the effect of machine environment and personal training on the performance of the hematological result?
2. What are the precision, accuracy and reliability of the hematology analyzer in the environment in which it is used?
3. How the accuracy, precision and reliability of result can be affected by some factors such as machine environment and personal training?
4. What is the effect of the duration of the use of the hematology analyzer on its performance?

1.4.2 General objective

The main objective of this study is risk assessment on auto hematology analyzer operation in addition to explore the relationship between the period of device work, environment and work condition on the performance of hematology analyzer.

1.4.3 Specific objective

1. To determine the performance of hematological analyzer results depends on machine environment and personal training.
2. To evaluate the accuracy, precision and reliability of results of hematological analyzer.
3. To correlate the accuracy, precision and reliability of results of hematological analyzer with factors such as machine environment and personal training.
4. To determine the installation of hematological analyzer.

1.5 Thesis layout:

This thesis consists of six chapters, chapter one discusses the problem definition, justification for carrying out the research and objectives.

Chapter two reviews some of the previous studies related to Assess the effect of pre analytical handling on Auto Hematology analyzer, Auto Hematology analyzer Performance evaluation, performance and some performance indicator definition.

The theory of Hematology analyzer (HA), blood formation, type of blood cells and problem happen due in increase or decrease or any abnormal deformation in blood cell, brief theory about blood cell counting, method of cell counting, type of automatic cell counter and finally focus on the mindray automatic cell counting which the instrument under research have been discuss in the chapter three and was selected due to availability of it the most medical laboratory.

Chapter four contains the methodology which includes materials, methods used, the data collection and analysis techniques.

Chapter five is results and discussions chapter six is conclusion recommendations.

CHAPTER TWO

LITERATURE REVIEW

LITERATURE REVIEW

2.1 Introduction

In the last decade, many research activities were conducted concerning Hematology analyzer which is useful to assessment and evaluate device before and after use, regarding it as a challenging and promising subject. it is observed that most study focus on sample stability and reliability of CBC parameters in different storage conditions ,comparison between two or more CBC of different manufacturer in addition to evaluate the performance of new device or method or evaluate a performance according to recommended document or guide line. Many factors can affect on analytical performance of hematology analyzer such as pre-analytical handling, time, and sample conditions in addition to the instrument used in the analysis whose results may be affected by some parameters.

Therefore, the following sections seek to review recent literature on assess the effect of pre analytical handling on Auto Hematology analyzer, Auto Hematology analyzer Performance evaluation as well as Auto Hematology analyzer performance indicator.

2.2 Assess the effect of pre analytical handling on auto hematology analyzer

Several studies have been conducted on assessing the effect of pre-analytical handling related to sample conditions which are respect to the time between blood drawing and the measurement and sample store temperature.

In a study of the sample stability for complete blood count using sysmex XN hematology analyzer by Daves, Massimo, et al. (2015) was conducted to investigate the reliability of hematological testing in samples stored for up to 24 h at different temperatures; No meaningful bias was observed after 3 h under different storage conditions, except for RDW and PLT-I (impedance technique, PLT-I) at 37 °C. After 6 h, meaningful bias was observed for MCH, MCV at room temperature, RBC count, (MCHC), MCH, MCV and PLT-I at 4 °C, and RBC, RDW, MCHC, MCH and PLT-I at 37 °C. After 24 h, a meaningful bias was observed for MCHC, MCV, platelet

count (fluorescent technique, PLT-F) and MPV at room temperature, MCHC, MCV, PLT-I and MPV at 4 °C, and all parameters except RBC count and MPV at 37.

Buoro et al, (30 may 2016) were done a study on assessment of blood stability for complete blood count using the sysmex XN-9000 and mindray BC-6800 analyzers at room temperature and 4°C up to 48 hour; RBC and reticulocyte parameters (i.e. HTC , MCV, MCHC ,RDW immature reticulocyte fractions, low-fluorescence reticulocytes, middle-fluorescence reticulocytes, high fluorescence mononuclear cells) showed less stability compared to WBC and PLT parameters (except for monocyte count and mean platelet volume). The bias for HTC, mcv, and RDW coefficient of variation was higher than the critical difference after 8h using both analyzers.it found that the blood sample measure with both analyzer do not shows analytical significant change up to 2 h of storage at room temperature and 4°C.As for the maximum time for analyses can be extend up to 8 h when the bias compared with critical differences.

A study that related to Reliability of Parameters of Complete Blood Count with Different Storage Conditions (Gunawardena, Dammika, et al, 2017) it evaluates samples storage up to 48 hrs. at temperatures of 4 ± 2°C, 23 ± 2°C, and 31 ± 2°C. Values were checked at time intervals of 6, 24, and 48 h ; t found CBC parameters, WBC, RBC, HGB, MCH, neutrophils and lymphocytes were stable at all three temperatures up to 48 hr. Monocytes, eosinophil, MCHC, HTC, and RDW-coefficient of variation showed statistically significant changes at 23 ± 2°C and 31 ± 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.it conclude that all CBC parameters are unaffected with the studied storage temperature up to 48 hr. except for the PLT which should be performed within 6 h of the post-collection time. To avoid changes in a few parameters such as Htc, it is best to store the sample at 4 ± 2°C if any delay is anticipated.

Wu, Dong-wen, et al (august 2017) were doing a reviewed study about how long can we store blood sample: systematic review and meta-analysis to assess the effect of storage time and temperature on complete blood count (CBC) and comprehensive metabolic panel (CMP) test; the result showed a total of 89 studies were confirmed .for CBC except MPV most parameters are stable at least for 24hr at

4°C .some indices such as WBC, HTC, HGB and MCH were stable up to 3 d at 4°C. Values were less stable when stored at RT.

Another study was done by Hussain, Sajid, et al (2018) about Evaluation and Comparison of Stability and Reliability of CBC Parameters Determined by Using Automatic Celltac G MEK-9100 Hematology Analyzer during Extended Storage at 4°C; the study showed WBC count was stable for up to 126 h, RBC and HGB levels were statistically stable for up to 186 h and 90 h. No significant changes were observed in NE, LY, MO, EO and BA for up to 42 h, 42 h, 66 h, 66 h, and 6 h respectively. PLT counts were stable for 6 h. Furthermore, results of HCT, MCV, MCH, MCHC, RDW-CV, RDW-S, PCT and MPV were statistically stable for up to 54 h, 42 h, 18 h, 30 h, 42 h, 30 h, 6 h and 6 h respectively. It deduced that it deduced that most parameters of CBC were unchanged till 48 h except for the PLT (6 h). To avoid changes in few parameters, such as MPV, basophiles, it is best to store the sample at 4°C if any delay is anticipated.

2.3 **Auto Hematology analyzer Performance evaluation**

In a study by Park IJ et al (august 2014) to evaluated the performance of The Samsung LABGEO (HC10) Hematology Analyzer in comparison to LH780 (Beckman Coulter Inc.), and also the differences due to the anticoagulant used. The result showed that the LABGEO has linearity over a wide range and a minimal carry over. The correlation between LABGEO (HC10) and LH780 was good for all complete blood cell count parameters, except for the mean corpuscular hemoglobin concentration. The bias estimated was acceptable for all parameters except for the monocyte count. The difference by anticoagulant type was statistically significant except for a few blood cell parameters.

A similar study evaluated the BC-3200 Automated Hematology analyzer and compared it with the Beckman- coulter AcT (AcT diff2) 3-part differential Hematology analyzer by L Peng et al (June -2008). The result demonstrated minimal carry over and excellent linearity for hemoglobin level (Hb), (WBC),(RBC),(PLT)(>0.998),precision was generally acceptable for all complete blood count parameters(CBC);coefficient of variation (CVs)were with in manufacture claims CVs of CBC parameters, Including RBC ,WBC ,and PLT, Hb and mean

corpuscular volume were (<6%). correlation between BC-3200 and Ac .T diff 2 was expellant(r>0.98) for all major CBC parameter (WBC ,RBC ,PLT count and Hb).they concluded that the overall performance of BC-3200 is excellent compare with coulter Ac .T diff 2.

Another study done by Tavany Elisa Santos Maciel,Samuel Ricardo Comar and Miriam Perlingeiro Beltrame (2014) to evaluate Sysmex (XE-2100D) analytical performance according to recommendations of the document H26-A2 of the Clinical and Laboratory Standards Institute (CLSI) .the results were satisfactory according to the manufacturer's specifications. The clinical sensitivity of the atypical lymphocytes flag showed efficiency, sensitivity and specificity of 92.5%, 65.2% and 94.1% respectively. The correlation coefficients between the automated and manual differential counts of neutrophils, lymphocytes, monocytes, eosinophil and basophils were 0.991, 0.99, 0.872, 0.974 and 0.557, respectively. They concluded that the Sysmex (XE-2100D) showed excellent analytical performance, and is useful to provide reliable hematology data.

Olga Ciepiela, Iwona Kotuła et al (2016) have done a study that aimed to comparison of Mindray BC-6800, Sysmex XN-2000, and Beckman Coulter LH750 Automated Hematology Analyzers using automated analysis of 807 anti-coagulated blood samples from children and 125 manual microscopic differentiations were performed. This comparative study included (WBCs, RBCs, and erythrocyte indices as well as platelet count). The study showed a poor level of agreement between WBCs enumeration and differentiation of the three automated hematology analyzers under comparison. A very good agreement was found when comparing manual blood smear and automated granulocytes, monocytes, and lymphocytes differentiation. Red blood cell evaluation showed better agreement than white blood cells between the studied analyzers.

Other study for Performance evaluation of Mindray CAL 8000(BC- 6800 and SC- 120) hematology analyzer and slide maker/strainer (Hwan Tae Lee, Pil-Whan Park et al, 2016). The performance of the BC- 6800 and Sysmex XE- 2100 were compared, and blood films by the SC- 120 and manual method were compared according to the CLSI guideline H26- A2 and H20- A2. The Results showed that most parameters measured by the BC- 6800 matched well with the XE- 2100 and manual

differential. The flag efficiency of the BC- 6800 for blasts (95.3%) and atypical lymphocytes (92.6%) were higher while immature granulocytes (89.7%) and NRBCs (94.1%) were lower than that of the XE- 2100. Additionally, the BC- 6800 detected four of five samples infected with plasmodium parasites. The SC- 120 showed no carry- over and expected repeatability. There was good agreement on the five- part differential including abnormal cells between blood films by the SC- 120 and manually prepared blood films. The shape of the RBC was also comparable between blood films.

Furthermore a Study was done by Noha Bassiouny Hassan Mostafa, Ayman Zakaria Ahmed Youssef and Mahmoud Abd Elzaher Kassab (2017) to evaluate the performance of NS-hema21t which is a new fully automated Egyptian hematology analyzer in terms of precision, trueness, linearity and carry-over, a comparison study with the Sysmex XT1800i and a manual reference leukocyte differential was performed. Flagging performance was also evaluated. The result showed excellent precision, trueness, linearity and carry-over results for all parameters tested. Comparison studies showed an acceptable correlation with both Sysmex XT1800i and the manual reference leukocyte count. A suboptimal flagging performance was demonstrated.

Moreover, another similar study entitled " Reproducibility of Hematological Parameters: Manual Versus Automated Method" was conducted by Aliyu A. Babadook, Isma'il M. Ibrahim et al (2019)to compare the blood count results of automated hematology analyzer with the traditional manual method in the determination of some hematological parameters .They concluded that The mean hematocrit, total white cell and platelets count, neutrophils, and lymphocytes percentages by manual method were 37.5 ± 7.2%, 7.2 ± 3.7 × 109/L, 244.8 ± 171.8 × 109/L, 53.8 ± 16.0%, and 41.8 ± 28.2%, respectively while that by automation were 37.2 ± 7.3%, 7.9 ± 6.1 × 109/L, 278.1 ± 162.0 × 109/L, 52.6 ± 16.0%, and 41.0 ± 14.3%, respectively. Whereas the mean platelets count was significantly ($P < 0.05$) higher in the automated method, there was no significant statistical difference between the mean hematocrit, total white cell and platelets count, neutrophils, and lymphocytes percentages of all the study samples ($P > 0.05$) and this remained so in male gender. The Pearson correlation test showed a positive significant ($P < 0.05$) correlation between both methods even after gender stratification.

A study about performance evaluation of the measurement of complete blood count parameters between mindray BC 6000 and BT PRO 2401 hematology analyzers was done by Saadet Kader(16 March 2020) which aimed to investigate the agreement between the results of complete blood count parameters values of that two instruments. he found that There was agreement between the two devices in WBC, Neu, Mon, Neuperc, Lymperc, Monperc, HCT, MCV, MCH, RDW SD, PLT, MPV and PCT parameters as interclass correlation coefficient (ICC) <90. However we found disagreement in Bas, Eos, Eosper, Basper, RBC, HGB,MCHC hemoglobin, RDWCV and PDW measurements between Mindray and BT-pro 2401 (ICC >90).

2.4 **Auto Hematology analyzer performance**

The CBC is measured daily in virtually all medical laboratories worldwide to screen for disease or abnormalities and in the follow-up of all kinds of medical therapies (surgery, transfusion, drugs, etcetera). The analytical results generated by HA are therefore the basis of numerous medical interventions, and it is importance that these results are analytically valid. It should be assured that the analytical performance is up to standard. To ensure the best quality of results hematologic laboratory testing there are many national and international organization operate to develop standards and guidelines intended to prevent discrepancies in laboratory testing and documentation such as WHO, FAD, ICHS(the international committee for standardization in laboratory) and CLSI (the clinical laboratory standards institute) which is focusing in pre analytical process, instrument validation by the manufacture, instrument evaluation by independent organization and instrument validation or /and verification by end user .

2.4.1 **Pre analytical process**

Pre analytical process is any producer that takes place before an analysis and any variable whose value can affect the outcome such as blood drawing process and all issue directly or indirectly related to this process.it starts from a physician's request for a laboratory test request to the preparation of a sample for testing, Patient preparation, sample collection, sample transportation, sample preparation, and sample storage. The most important pre analytical variable for the CBC is time because times dependent alternations on CBC parameters may occur because CBC is measured on viable blood cells undergo time dependent degenerative process. the most

remarkable time dependent change include change in blood cell volume such as MCV, MPV and WBC differentiate cause by morphological changes that can be observe by microscope. Therefore the sample test should occur within 4 to 8 hours after venipuncture when the sample store at room temperature. (Scoffin, 2014)

2.4.2 Instrument validation/evaluation

It is a series of processes through which you test your system to verify or validate the performance specifications published by the manufacturer of the instrument. It is validate the manufacturer's claims for their method performance characteristics, under your current environmental conditions, e.g. temp, humidity, water, electricity, operator skills etc. In addition to ensure that the amount of error present in the system won't affect the interpretation of the test result and compromise patient care and Ensure that effects of shipment and storage did not affect your instrument performance. There are several stages of evaluation before using analyzer in the laboratory.

2.4.3 Instrument validation by the manufacturer

It's define as all the action or process of proving that the producer, process, system, equipment or method used works as expected and achieves the intended result. It required by CLIA regulation and the manufacture of HA is responsible for setting objective for the analytical application and performance of instrument. The objective should be investigated using well defined testing protocols and there performance validated against either an accepted reference method or an on market analyzer if there is no reference methodology .the general parameters assess are: back ground, carry over ,imprecision ,comparability ,lower limit detection and interference. In addition to that the CLIA and ICSH guideline as well as ISO accreditation regulation state that the responsibility of instrument installation setup and initial calibration using specific method lie on manufacturer.

2.4.4 Instrument evaluation by independent organization

According to the ICSH standards, new instrument could be evaluate by independent national organization following ICSH guidelines or the end user has to perform a less extensive evalution.in countries lacking these official organizations the end user laboratory can evaluate instrument by seeking counsel in evaluations published in peer reviewed journals and to determine its own extent of validation and or verification strategy depending on its own objective .Evaluation should include in-

strument installation, user application, blood sample and instrumentation evaluation .(Verbrugg , Huisman ,2015)

2.4.5 Instrument validation or /and verification by end user

Last validation of instrument to assess the manufacturer claim on performance of specific instrument should do by the end user applying intended use criteria by the laboratory.it include performance analysis of an HA on accuracy ,normal range ,back ground, carry over ,lower limit of detection, quantitation ,clinical reportable in travel and linearity.(Verbrugg , Huisman , 2015)

2.5 Auto hematology analyzer performance indicator definition

2.5.1 Precision

Precision is the degree of reproducibility among several independent measurements of the same sample for the same analyze .Precision experiment is performed to estimate the imprecision or random error of the analytical method. It is measured in terms of coefficient of variation (CV) and standard deviation (SD). The smaller the CV and SD mean the better the precision. (VIS, HUISMAN, 2016)

2.5.2 Accuracy

A measurement of the exactness of an analytical method, or the closeness of agreement between the measured value and the true value.it Performed to estimate inaccuracy or systematic error of the new method. Experiment is performed by analyzing elven or more patient samples by the new method (test method) and a comparative method, then estimate the systematic errors on the basis of the differences observed between the two methods .(VIS, HUISMAN ,2016)

2.5.3 Back ground

Background refers to any signal (noise) that is measured but does not originate from the parameter of interest. Background may be caused by an interfering substance, for example, signals from blood free reagents or electronic noise caused by the HA. The background counts should be zero or very low cell counts. (VIS, HUISMAN, 2016)

2.5.4 **Carry over**

Carryover is defined as the amount of analyze carried by the HA from one sample measurement into the subsequent measurement .it may erroneously affect the reported concentration in the subsequent sample. (VIS, HUISMAN, 2016)

2.5.5 **Comparability**

It is comparability study to compare many samples using HA. It is importance to include normal and abnormal samples in approximate equal proportions.it use to evaluate two or more HA or new HA .The difference between the HA under evaluation and the current analyzer or reference method should be as low as possible and The parameter from the HA under evaluation can be compared with the current HA using linear regression. (VIS, HUISMAN, 2016)

2.5.6 **Linearity**

Linearity is an assessment of the lowest and highest levels at which an analytic can be accurately measured without any type of dilution or concentration. It is important to validate the manufacturer's claims for reportable range of their system or method. (VIS, HUISMAN, 2016)

2.6 **Reference range of complete blood count**

WuX, ZhaoM and et.al (2015) were done a study about complete blood count reference intervals for healthy Han Chinese adults. For 4642 healthy individuals from six clinical centers in China. The Results Median and mean platelet counts from the Chengdu center were significantly lower than those from other centers. RBC, HGB, and HCT values were higher in males than in females at all ages. Other CBC parameters showed no significant instrument, region, age, or sex-dependent difference. Thalassemia carriers were found to affect the lower or upper limit of different RBC profiles. It concluded to establish consensus intervals for CBC parameters in healthy Han Chinese adults. RBC, HGB, and HCT intervals were established for each sex. The reference interval for platelets for the Chengdu center should be established independently.

Another study of Complete Blood Count Reference Intervals and Patterns of Changes across Pediatric, Adult, and Geriatric Ages in Korea done by Nah ,Eun-Hee

and et al (2018). The CBC parameters RBC, WBC, and PLT from 781,857 examinees were studied. We determined statistically significant partitions of age and sex, and calculated RIs according to the CLSI C28-A3 guidelines. It concluded that the CBC parameters show dynamic changes with both age and sex.

Similar study about reference ranges of white blood cells count among Sudanese healthy adults done by Taha, Elmutaz H and et al (2018). For 1076 healthy Sudanese adults from both sexes and five states, with age range of 20 – 60 years. Clinical examination was performed, weight and height were measured, and BMI was calculated. Blood samples were obtained from brachial veins and drawn in EDTA tubes. The results showed that WBCs count was positively correlated with BMI. The count was found to be significantly higher in Red sea and Darfur states compared with the other states. The reference ranges of WBCs count in Sudanese are lower than the international one and should be used in Sudan.

Another study for reference values for hemoglobin and red blood cells indices in Sudanese in Khartoum state was done by Awad, Kamal M and et al (2019). A total of 438 healthy adults between 20 and 60 years resident in the Khartoum state (90 males and 348 females) were included. A complete blood count (CBC) was performed for Hb, RBCs, PCV, MCH, MCV and MCH Cussing Sysmex KX-21 automated hematology analyzer. It Concluded to Some CBC reference values like Hb level in Khartoum state were lower than the international values.

2.7 **Conclusion**

Assessment the effect of some parameter in the diagnostic and therapeutic instruments is very important issue to avoid Clinical incidents which can lead to wrong medication and patient can passed away .the goal of this review was to search about the effect some parameters hematological analyzer results. The reviewed literature show that most studies focus on assessing the effect of pre-analytical handling related to sample conditions (time between blood drawing and the measurement and sample store temperature) and other studies concentrated on evaluate the performance of auto hematology analyzer or comparison of two or more of it with respect to manufacture claims, reliability, linearity, carry over, precision, sensitivity, specify and correlation between compared instrument and manual blood smear. In addition

to assessed the performance according to recommended document. Current research assess the effect of machine environment and personal training on the auto hematological analyzer results and the accuracy, precision and reliability correlated to the running period of the instrument which is a new type of study and there are no studies before it, even in Sudan.

CHAPTER THREE

THEORETICAL BACKGROUND

THEORETICAL BACKGROU

3.1 Introduction:

This chapter of the thesis will look into the theory of Hematology analyzer (HA) which is used to count and identify blood cells at high speed and accuracy.it concentrate on blood formation ,type of blood cells and mention some problem happen due in increase or decrease or any abnormal deformation in blood cell.in addition to brief theory about blood cell counting ,method of cell counting ,type of automatic cell counter and finally focus on the mindray automatic cell counting which is the instrument under research

3.2 Blood:

3.2.1 Definition of blood:

Blood is a type of connective tissue .it is circulated through the heart ,arties ,veins and capillaries carrying nourishment ,electrolyte hormones vitamins ,antibodies ,heat and oxygen to body tissue and taking away waste matter and Carbone dioxide .(Merghani,2010; Joseph Ed,D Bronzing,2000).

It consists of cells and intercellular substance .the cells are red blood cells (RBCs) or erythrocytes, white blood cells (WBCs) or leukocytes and platelets or thrombocytes .the intercellular substance is called plasma. Volume of blood is about 5 litters in an average adult male it equals 8% of total body weight and volume of plasma is about 3.5 litters it equals 5% of the total body weight. (Merghani, 2010)

3.2.2 Functions of blood:

1. Transport of gases (e.g. oxygen and carbon d dioxide), nutrient (e.g. glucose, amino and free fatty acid), waste products (e.g. Urea and uric acid) and hormones (e.g. catecholamine, insulin, cortisol and thyroid hormones). (Merghani,2010)
2. Defense by WBCs.
3. It maintains the constancy of the internal environment which is the extracellular fluid .it controls temperature by distribution of the heat by blood and con-

trol of PH by buffers in the blood e.g. $HCO3^{-}$, proteins and hemoglobin this process called Homeostasis.

4. Blood loss is prevented by arrest of bleeding (e.g. by platelet and clotting factors) and Stander check of blood in fluid state by the natural anticoagulants this process called Hemostasis. (Merghani,2010)

3.2.3 **Hematopoiesis:**

It is the process of blood cell production, differentiation, and development. The hematopoietic system consists of the bone marrow, liver, spleen, lymph nodes, and thymus. It starts as early as the 3rd week of gestation in the yolk sac. By the 2nd month, hematopoiesis is established in the liver and continuous through the 2nd trimester. During the 3rd trimester it shifts gradually to bone marrow cavities. During infancy: all marrow cavities are active in erythropoiesis "Red Marrow". During childhood: erythropoiesis becomes gradually restricted to flat bones as; skull, vertebrae, sternum, Ribs and pelvic bones, in addition to ends of long bones. The shafts of long bones become populated by fat "yellow marrow". About the 75% of the cells in the red bone marrow are WBC precursors and 25 % are RBC precursors even though the RBC count is over 500 times more than the WBC count this indicates the longer life span of RBCs.(Merghani,2010)

3.2.4 **Blood Cell Development:**

The pluripotent stem cell is the first in a sequence of steps of hematopoietic cell generation and maturation. The progenitor of all blood cells is called the multipotential hematopoietic stem cell. These cells have the capacity for self-renewal as well as proliferation and differentiation into progenitor cells committed to one specific cell line.

The multi-potential stem cell is the progenitor for two major ancestral cell lines: Lymphocytic and non-lymphocytic cells. The lymphoid stem cell is the precursor of mature T cells or B cells/ plasma cells. The non-lymphocytic (myeloid) stem cell is progress to the progenitor CFU-GEMM (colony-forming unit granulocyte-erythrocyte-monocyte-megakaryocyte). The CFU-GEMM can lead to the formation of CFU-GM (CFU-granulocyte-macrophage / monocyte), CFU-Eo (CFU-Eosinophil), CFU-Bs (CFU-basophil) and CFU-MEG (CFU-Megakaryocyte). In erythropoiesis,

the CFU-GEMM differentiates, into the BFU-E (Burst-Forming unit Erythroid). Each of the CFUs in turn can produce a colony of one hematopoietic lineage under appropriate growth conditions. CFU-E is the target cells for erythropoietin. Figure 3.1 shows the divisions of the pluri potential cells to form the different blood cells.

Growth and reproduction of the different stem cells controlled by hematopoietic growth factors .The hematopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of hematopoietic progenitor cells and the function of mature blood cells. These growth factors interact with blood cells at different levels in the cascade of cell differentiation from the multi potential progenitor to the circulating mature cell. (Abdul Hamid, 2012; Merghani, 2010)

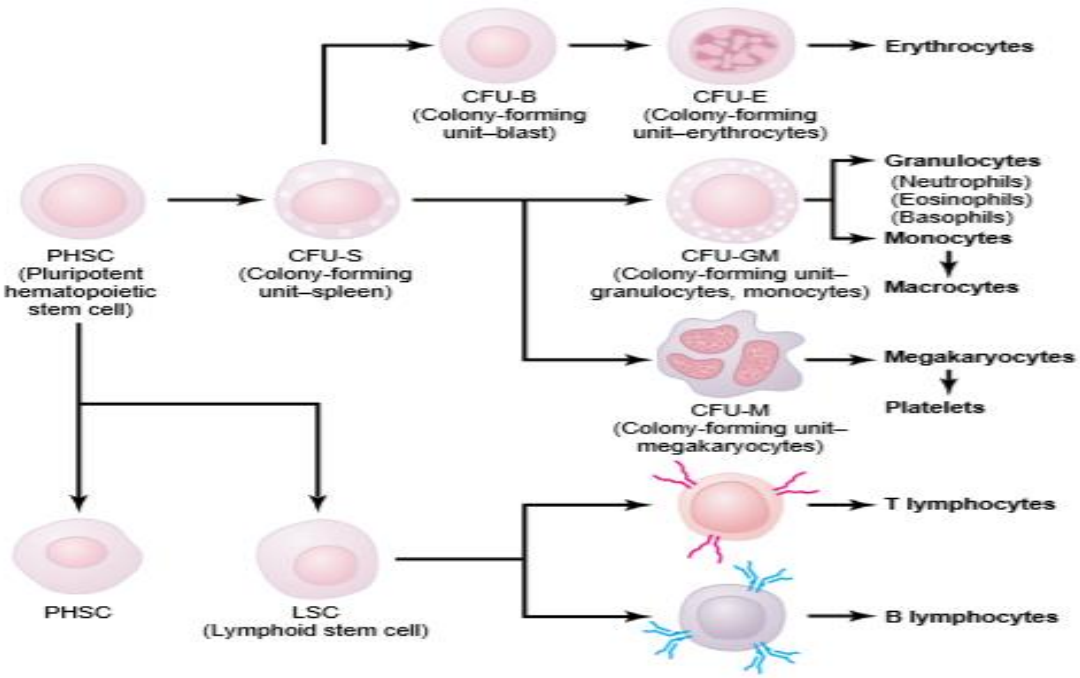


Figure 3.1 Formations of different blood cells from the original pluripotent hematopoietic stem cell (PHSC) in the bone marrow

3.2.5 **The Red Blood Cells (erythrocyte)**

3.2.5.1 **Stages of erythropoiesis**

Erythropoiesis is the process of erythrocyte differentiation, and development. It can be divided into stages in the bone marrow and stages in the blood. Erythrocytes are rapidly maturing cells that undergo several mitotic divisions during the maturation process.

In the stage of the bone marrow which called replication phase .In this stage the numbers of RBCs increase .The CFU-E stem cells divide to form nucleated cells known as Proerythroblast or (normoblast). Proerythroblast is the first identifiable cell of this line followed by the Basophil erythroblast "this cell have very little hemoglobin, polychromatic erythroblast and orthochromatic erythroblast in these generation the cell become filled with hemoglobin to a concentration about 34% .the Proerythroblast loss the nucleus and other organelles to become reticulocytes .

In the stage of blood which called maturation phase. In the reticulocyte stages which is the generation before become mature erythrocyte the cell pass from bone marrow into blood capillary by diapedesis (squeezing through the pores of the capillary membrane. Reticulocytes enter the circulating blood and fully mature into functioned erythrocytes. .in this stage the size of RBCs decrease and loss nucleus and organelles Figure 3.2 shows RBCs formation stages. (HALL, GUYTON, 2006).

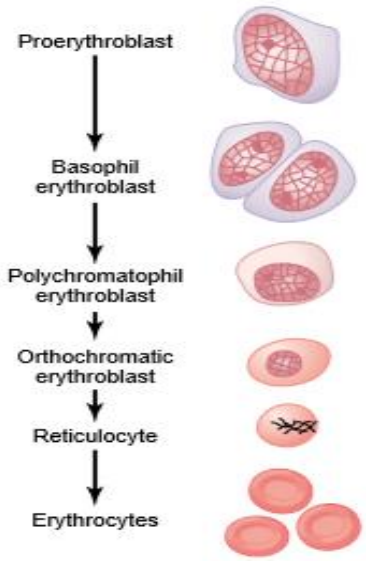


Figure 3.2 Stages of formation normal red blood cells

3.2.5.2 **Characteristics of RBCs:**

1. Their Life span is about 120 days before being destroyed. (HALL, GUYTON, 2006).
2. They do not have a nucleus therefore no reproduction.(Merghani ,2010)
3. They do not have mitochondria, or endoplasmic reticulum, they do have cytoplasmic enzymes that are capable of metabolizing glucose to form small amounts of adenosine triphosphate for maintenance of the integrity of the cell membrane.(Merghani ,2010)
4. Shapes of normal RBCs are biconcave discs. Show figure 3.3.(Merghani ,2010)
5. Size of normal RBCs :show figure 3.3

   Diameter is about 7.5 micrometers.

   Thickness is about 2.0 micrometers at the thickest point and 1 micrometer or less in the center. (Merghani, 2010)
6. The average volume of the red blood cell is 90 to 95 cubic micrometers. (HALL, GUYTON, 2006).
7. Contain hemoglobin which is a red pigment found within RBCs. (HALL, GUYTON, 2006).
8. Shapes of RBCs can change remarkably as the cells squeeze through capillaries. (HALL, GUYTON, 2006).
9. Count of RBCs: in normal male the average number of red blood cells per cubic millimeter is 5,200,000 (±300,000); in normal female, it is 4,700,000 (±300,000). (HALL, GUYTON, 2006).

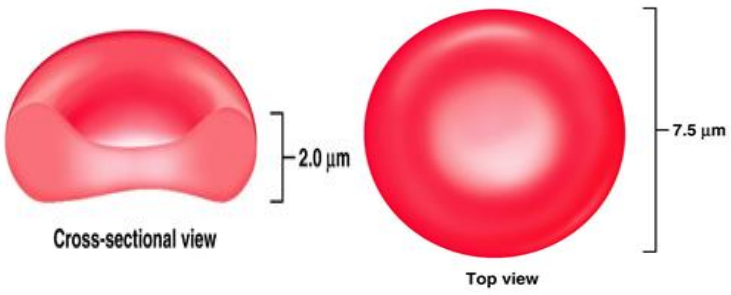


Figure 3.3 Size and shape of normal RBCs

3.2.5.3 **Functions of RBCs:**

The main function of RBCs is to transport hemoglobin, which in turn to carry oxygen from lung to tissues. (HALL, GUYTON, 2006).

1. To transport carbon dioxide (CO_2) in the form of bicarbonate ion (HCO_3^-) from the tissue to lung where it reconverted to CO_2 and expelled into the atmosphere. (HALL, GUYTON, 2006).
2. The RBCs are responsible for the most of the acid base buffering power of whole blood because the hemoglobin is an excellent acid base Buffer. (HALL, GUYTON, 2006).
3. Contain antigens on its surface that determine the type of blood group. (HALL, GUYTON, 2006).

3.2.5.4 **Problems with RBCs**

1.Anemia

Anemia is a decrease in red cell mass is also defined as a decrease in the hemoglobin concentration or decrease in the hematocrit when compared with a normal group. Functionally define as a decrease in the competence of blood to carry oxygen to tissues thereby causing tissue hypoxia.

The symptoms of anemia depend upon the degree of reduction in the oxygen-carrying capacity of the blood, the change in the total blood volume, the rate at which these changes occurs, the degree of severity of the underlying disease contributing to the anemia, and the power of the cardiovascular and hematopoietic systems to recuperate and compensate.

To understand anemia, it is necessary to understand normal erythrocyte kinetics. Total erythrocyte mass in the steady state is equal to the number of new RBCs produced per day times the erythrocyte life span (100-120 days).

$$\text{Mass (M)} = \text{Production (p)} \times \text{Survival (s)} \quad \ldots\ldots\ldots\ldots\ldots (3.1)$$

For example: if the average 70-Kg man with 2 liters of erythrocytes must produce 20 ml of new erythrocytes each day to replace the 20 ml per day normally lost due to cell senescence.

$$2000 \text{ ml (M)} / 100\text{days(S)} = 20 \text{ ml/day (p)}$$

If the survival time of the erythrocyte is decreased by half, the bone marrow must double production to maintain a constant mass.

2000ml (M) / 50days(S) = 40 ml/day (P)

The marrow can compensate for decreased survival in this manner until production is increased to a level 5 to 10 times normal, which is the maximal functional capacity of the marrow. (Abdul Hamid, 2012)

Some types of anemia and their physiologic causes are the following:

1. Microcytic hypochromic anemia: it causes of blood loss and iron deficiency which lead to low hemoglobin, small and pale RBCs. (Abdul Hamid, 2012; HALL, GUYTON, 2006).
2. Macrocytic normochromic anemia (Megaloblastic Anemia): it causes of malnutrition, decrease absorption of folic acid and vitamin B12 and increased requirement of folic acid and vitamin B12 due to pregnancy or drugs. (Abdul Hamid, 2012; HALL, GUYTON, 2006).

   Anemia laboratory investigations:

   1. RBC count, Hematocrit and Hemoglobin.
   2. Red Cell Indices.
   3. Reticulocytes count.
   4. Blood smear examination.
   5. Bone marrow examination. (Abdul Hamid ,2012)

3. Polycythemia

Polycythemia (Erythrocytosis) is excess erythropoietin .it is opposite of anemia. It is an increase in red cell mass is also defined as an increase in the hemoglobin concentration or increase in the hematocrit when compared with a normal group. It leads to increase viscosity of the blood which decreases the rate of venous return to the heart and blood flow become very sluggish .in addition to increase the blood volume.

Polycythemia caused by many reasons:

1. Renal problem (e.g. renal tumor or polycystic kidney disease by excessive release of erythropoietin).
2. Chronic respiratory problem these problem cause hypoxia.

3.Patient develops polycythemia (e.g. lung fibrosis).

4.People live at altitudes of 14,000 to 17,000 feet, where the atmospheric oxygen is very low which called physiologic polycythemia. (HALL, GUYTON, 2006).

3.2.6 **The white blood cells (leukocytes):**

The white blood cells (WBCs) or leukocytes are the mobile units of the body's protective system .they are transported in the blood to different parts of the body where they are needed. It works in two ways to protect the body against invaders:

1.By destroying invading bacteria or viruses by phagocytosis.

2.By forming antibodies and sensitized lymphocytes. (HALL, GUYTON, 2006).

There are five types Classified into two main types granulocytes and a granulocytes depend on presence or absences of granular in their cytoplasm. The granulocytes have granulated cytoplasm, ploy segmented nucleus and very short life span .it is also have three types according to reaction of the cytoplasmic granules with acidic or basic dye .these types are neutrophils, basophiles and Eosinophils. As for granulocytes have non –granulated cytoplasm, one nucleus and longer life span .it classifies to two types monocytes and lymphocytes. (Merghani, 2010)

3.2.6.1 **Stages of leucopoiesis:**

Leucopoiesis is the process of leukocytes formation. The formation site divided to bone marrow, thymus, lymph node and lymph tissue. The granulocytes and monocyte form in the bone marrow as for lymphocyte and plasma form in the bone marrow, thymus, lymph node and lymph tissue. The leucocyte formation is divided into two lineages myelocytic and lymphocytic lineage. The WBCs formed in the bone marrow are stored within the marrow until they are needed in the circulatory system. The lymphocytes are mostly stored in the various lymphoid tissues, except for a small number that are temporarily being transported in the blood. (HALL, GUYTON, 2006).

Formation of granulocytes begins in the bone marrow where there is progressive division and maturation. The first identifiable stem cell in granulocytic series is myoblast, the next stage is pro myelyocyte followed by meylocyte, metamyelyocyte then division and maturation granulocytes. Two stages of granulocytes are observed

in the circulating blood: the band form of neutrophils, eosinophils and basophils and in end stage of maturation (Abdul Hamid,2012) figure 3.4 shows granulocytes formations.Formation of a granulocyte start from the primitive unipotent cell (lymphoid-committed precursor) in the thymus, lymphoid tissue and bone row .lymphocyte production pathway includes the following stages. The first stage is lymphoblast flow by intermediate (transitional) forms large blast cell then small and large lymphocyte. (Mohamed Yousif Sukker, et al, 2000). Figure 3.5 shows stages of lymphocyte formation.

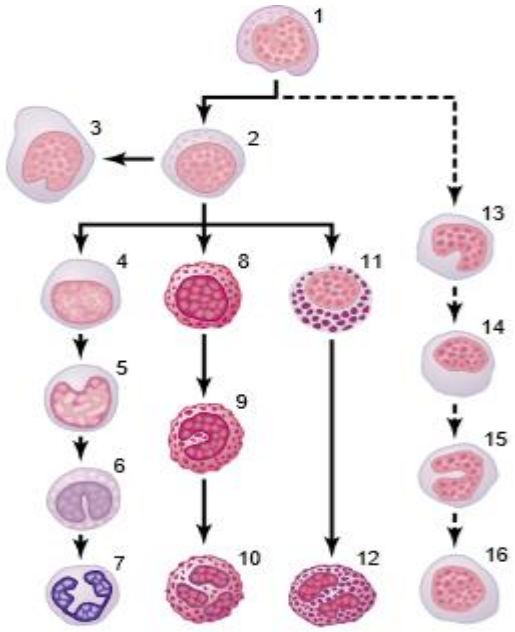


Figure 3.4 Diagram of the process of granulocyte formation.

(1) Myeloblast, (2) promyeloblast, (3) megakaryocyte, (4) neutrophil myelocyte, (5)young neutrophil metamyelocyte,(6) “band” neutrophil metamyelocyte (7)polymorphonuclear neutrophil,(8) eosinophil myelocyte,(9)eosinophilmetamyelocyte,(10)polymorphonucleareosinophil,(11)Basophilmyelocyte,(12)polymorphonuclear basophil,(13-16)stages of monocyte formation.(HALL,GUYTON ,2006).

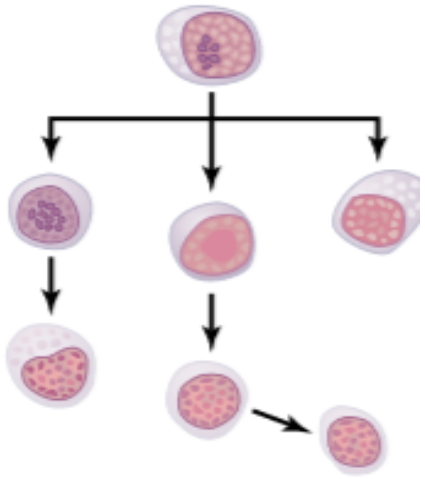
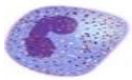
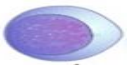
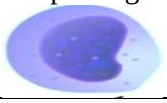


Figure 3.5 Diagram of the process of lymphocyte formation

3.2.6.2 **Characteristics of WBCs:**

1. They have nucleus.(Merghani ,2010)
2. They do not have hemoglobin.(Merghani ,2010)
3. Life span of the granulocytes are normally 4 to 8 hours in the blood and 4 to 5 days in tissues but in cause of tissue infection his total life span is often shortened to only a few hours, monocytes also have a short transit time, 10 to 20 hours in the blood as for the lymphocytes have life spans of weeks or months depends on the body's need for these cells. (HALL, GUYTON, 2006).
4. Count of WBCs: in normal human being about 7000 white blood cells per microliter of blood.
5. WBCs enter the tissue Spaces by diapedesis. Neutrophils and monocytes can squeeze through the pores of the blood capillaries even though a pore is much smaller than a cell. (HALL, GUYTON, 2006).
6. WBCs move through tissue spaces by ameboid motion.[4]
7. Table 3.1 below shows size, shape and count of WBCs in normal person: (Sucker, et al, 2000).

Table 3.1 The size, shape and count of WBCs in normal person

Type of WBCs	%byvolume of WBCs	Count (number of cells /mm3)	Size	Shape	Life span
Neutrophils	40% - 62%	3000-7000	10µm - 16µm	▪ Nucleus has many interconnected lobes. ▪ Nucleus is made of denes masses which take the purple stain. ▪ Purple granules. ▪ Cytoplasm contains fine granules which stain purplish. 	60-72 Hours
Eosinophils	0.5%- 1%	100-400	12µm - 18µm	▪ Nucleus has bi-lobes nuclei. ▪ Nucleus is made of stains less deeply than neutrophil. ▪ Bright red granules. ▪ Cytoplasm contains large spherical granules. 	60-12 Days
Basophiles	0.5%- 1%	20-50	10µm - 14µm	▪ Bilobed nuclei hidden by large granules . ▪ Dark blue granules. ▪ cytoplasm contain largecorse rounded or oval daeg deeply staining granules which overlie the nucleus . 	4-72 Hours
Lymphocytes	20%-40%	1500-3000	5µm-8µm (small) Or 9µm-15µm (medium and large)	▪ Rounded nucles . ▪ Large type has abundant cytoplasm which usaullay takes pale blue stain and may cntain reddish granules . ▪ small type has a very scanty cytoplasm forming small rim around the nucleus. 	Tcells: 100-300days or Even more B cells : 2-7days
Monocytes	6% -10%	100-700	15µm - 20µm	▪ Kideny shped nucleus . ▪ No granules. ▪ ytoplasm is grey –blue giving aground glass appearance with fine reddish azurphilic granules. 	Several months

3.2.6.3 **Functions of WBCs**

1. Protect the body against invaders. (HALL, GUYTON, 2006).
2. Neutrophils and tissue macrophages attack and destroy invading bacteria, viruses, and other injurious agent. WBCs are attracted to Inflamed Tissue Areas by Chemotaxis which is the chemical substance. (HALL, GUYTON, 2006).
3. Phagocytosis which means cellular ingestion of the offending agent by neutrophils and macrophages. (HALL, GUYTON, 2006).
4. Forming antibodies and sensitized. (HALL, GUYTON, 2006).

3.2.6.4 **Problem with WBC**

1. Leukocytosis

Leukocytosis is an increase in the total leucocyte count above the normal.it may occur in health and disease. Health leukocytosis also called physiological leukocytosis occurs due to several conditions. These conditions are:

1. Diurnal variation (leukocyte count are decrease in the morning and increase in the afternoon).
2. After a protein meal
3. Following physical exercise.
4. Stimulation by stress or injection of adrenaline.

As for disease leukocytosis it cause due to bacterial infections.in this cases measurement of total leukocyte count is essential for diagnosing the existing of the infection.in general acute bacterial infections cause increase in the neutrophil count while chronic and viral infections it increase lymphocyte count. (Mohamed Yousif Sukker, et al, 2000).

2. Leucopenia

Leucopenia is a decrease in the total leucocyte count below the normal.it happen due to malnutrition and also it is a feature of typhoid fever.in addition to some drugs may depress the bone marrow or deficiency of vitamin B12 or folic acid. Therefor leucopenia causes particularly a decrease in granulocyte count (a granulocyte). (Mohamed Yousif Suckker, et al, 2000).

3. Leukemias

Uncontrolled production of white blood cells can be caused by cancerous mutation of a myelogenous or lymphogenous cell. This causes leukemia, which is usually characterized by greatly increased numbers of abnormal white blood cells in the circulating blood.it is divided into two general types: lymphocytic Leukemias and myelogenous leukemia (HALL, GUYTON, 2006). the total white blood cell is usually very high (>50000mm3).the leucocyte precursors in the bone marrow proliferate extensively and occupy bone marrow space which result in a concomitant depression in the production of red cells ,leading to anemia and platelet leading to thrombocytopenia. (Mohamed Yousif Sukker, et al, 2000).

3.2.7 **Platelet (thrombocytes)**

3.2.7.1 **Stages of thrombopoiesi**

Thrombopoiesis is the process of thrombocytes formation. They are formed in the bone marrow from the megakaryoblast, mature through the stages of promegakaryocytes and megakaryocytes. Megakaryocytes are extremely large cells of the hematopoietic series in the marrow, the megakaryocytes fragment into the minute platelets either in the bone marrow or soon after entering the blood, especially as they squeeze through capillaries. (Mohamed Yousif Sukker, et al, 2000).

3.2.7.2 **Characteristic of platelet**

1. Platelets are minute discs 2to 3 micrometers in diameter. (Mohamed Yousif Sukker, et al, 2000).
2. The normal concentration of platelets in the blood is between 100,000 and 400,000 per microliter. (Mohamed Yousif Sukker, et al, 2000).
3. It does not have nuclei and cannot reproduce. (Mohamed Yousif Sukker, et al, 2000).
4. It half-life in the blood of 3 to 5 days. (Mohamed Yousif Sukker, et al, 2000).

3.2.7.3 **Function of platelet**

1. Vasospasm .they releases a vasoconstrictor. (Merghani ,2010)
2. Platelet plug. (Merghani ,2010)
3. Blood coagulation (it release some clotting factors) .(Merghani ,2010)

3.2.7.4 **Problem with platelet**

1. Thrombocytopenia

Platelet deficiency result in increased bleeding tendency .it a count lessthan50000 cell/µl. associated with hemorrhage after minor injuries and possibly multiple petechial hemorrhages under the skin and mucus membranes (thrombocytopenic purpura) .Purpura is very common when platelet counts is less than 20000 cell/µl. (Merghani, 2010)

2. Thrombocytosis

It happens when platelet count is high due thrombotic problems. (Merghani, 2010)

3. Hemophilia

Hemophilia is a bleeding disease.it is caused by an abnormality or deficiency of factor VIII or factor IX. (HALL, GUYTON, 2006).

3.2.8 **Plasma**

Plasma is the fluid part of the body .it about 5%of the total body weight in a 70kg adult male (3.5L). (Merghani, 2010)

3.2.8.1 **Plasma formation**

Most of the plasma proteins (albumin, globulins α and β) are synthesis in the liver except gamma globulin which synthesis in the reticuloendothlial system by plasma cell. (Mohamed Yousif Sukker, et al, 2000).

3.2.8.2 **Characteristic of plasma**

1. It contains 91% water and 9%solid which are proteins, inorganic salts (sodium and bicarbonate) and organic substance (fat, glucose, bilirubin, urea…).(Mohamed Yousif Sukker, et al,2000).
2. Plasma protein divided into three types (albumin, globulins, and fibrinogen). (Mohamed Yousif Sukker, et al, 2000).
3. Half-life of albumin is 20days. (Mohamed Yousif Sukker, et al, 2000).
4. Total plasma concentration 6-8 g/dl.(Merghani,2010)

3.2.8.3 **Function of plasma**

1. Transport functions (α-and β-goblins). (Mohamed Yousif Sukker, et al, 2000).
2. Defensives (immunoglobulin). (Mohamed Yousif Sukker, et al, 2000).
3. Reverse of body protein. (Mohamed Yousif Sukker, et al, 2000).
4. Osmatic function (albumin) through control of the exchanging of fluid between blood and tissue. (Mohamed Yousif Sukker, et al, 2000).
5. Viscosity of plasma is due mainly to fibrinogen a globulin. (Mohamed Yousif Sukker, et al, 2000).
6. Fibrinogen is the precursor of fibrin in the blood clot. Prothrombin in a α_2– globulin and most of the remaining clotting factors are β –globulins. (Mohamed Yousif Sukker, et al, 2000).
7. Buffer. (Merghani,2010)

3.2.8.4 **Problem with plasma**

1. Hypoalbuminaemia

It occurs when the concentration of plasma albumin less than 2g/100ml due to malnutrition, liver (gross damage liver cells) disease and kidney disease (nephritic syndrome). (Mohamed Yousif Sukker, et al, 2000).

2. Haemoglobinuria

It occurs when hemoglobin level exceed haptoglobin binding ty .hapatoglobins are a group of globulins which have property of binding free hemoglobin in the plasma. (Mohamed Yousif Sukker, et al, 2000).

3.3 Blood cell counter

Blood cell counter uses to measure the number of red blood cells, white blood cells, and platelets in the blood. The amount of hemoglobin and the hematocrit are also measured. It helps in diagnose and monitor many conditions. As known, the Changing in the normal functioning of an organism is often accompanied by changes in the blood cell count. Therefore, the determination of the number and size of blood cells per unit volume often provides valuable information for accurate diagnosis.

3.3.1 **Methods of cell counting**

The blood cell counter counts the number of RBC or WBC per unit of volume of blood using either of two methods: manual method and automatic method.

3.3.1.1 **Manual cell counting method**

One of the most common and routinely method of counting blood cells even today is the microscopic method which known as the counting chamber technique. In this method the sample is diluted then visually examined and the cells counted. It uses two gridded chambers which are covered with a special glass slide when counting. A drop of blood is placed in the space between the chamber and the glass cover then Looking at the sample under the microscope and uses the grid to manually count the number of cells in a certain area of known size. The separating distance between the chamber and the cover is predefined, thus the volume of the counted culture can be calculated and with it the concentration of cells. This method is suffers from several drawbacks which are:

1. A Subjective and system errors which are lead to poor reproducibility of the results.
2. The lengthy procedure involved results in the rapid tiring of the person making the examination.
3. Poor time and labor utilization.
4. The data gathered by this measurement is not directly suitable for storage or for further processing and evaluation.(Raghbir Singh Kandpur,2003)

3.3.1.2 **Automatic cell counting methods**

1. Optical method counting cell

Optical method is based on collecting scattered light from the blood cells and converting it into electrical pulses for counting. Figure 3.6 shows one type of the rapid counting of red and white cells using the optical detection system. A sample of dilute blood (1:500 for white cells and 1:50,000 for red cells) is taken in a glass container. It is drawn through a counting chamber in which the blood stream is reduced in cross-section by a concentric high velocity liquid sheath. A sample optical system provides a dark field illuminated zone on the stream and the light scattered in the forward direction is collected on the cathode of a photomultiplier tube. Pulses are

produced in the photomultiplier tube corresponding to each cell. These signals are amplified in a high input impedance amplifier and fed to an adjustable amplitude discriminator. The discriminator provides pulses of equal amplitude, which are used to drive a digital display. Instruments based on this technique take about 30 s for completing the count. An accuracy of 2% is attainable. The instruments require about 1 ml of blood sample. (Raghbir Singh Kandpur, 2003)

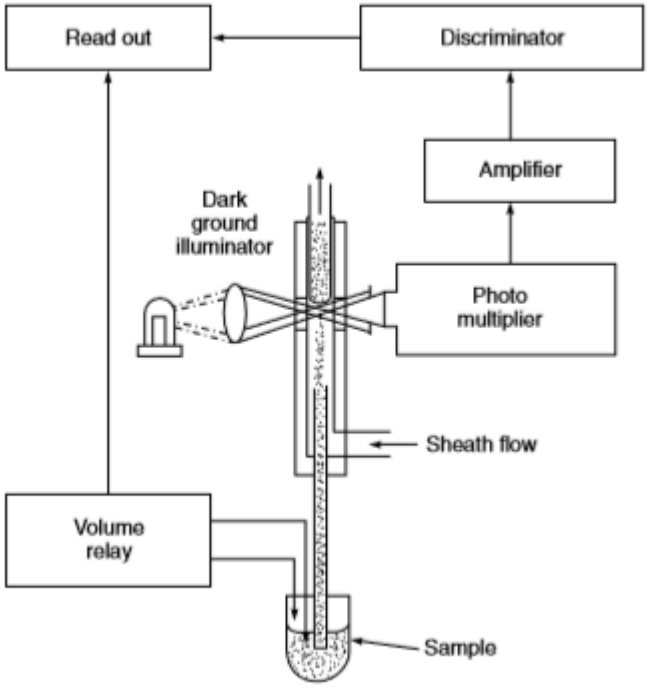


Figure 3.6 Optical method of counting cell

2. Electrical conductive method (coulter method):

The principle of conductivity change happens when cell pass through an aperture which known as Coulter Counters (electrical impedance). It is basis of several counting instruments manufactured by a number of manufacturers throughout the world. The technique is useful for determining the number and size of the particles suspended in an electrically conductive liquid. The principle of measurement is built depend on the blood which is a poor conductor of electricity but a certain diluents are good conductors. Therefore, blood is diluted and the suspension is drawn through a small aperture to count cell. By means of a constant current source, a direct current is maintained between two electrodes located on either side of the aperture. When a blood cell is passing through the aperture, it displaces some of the conductive fluid

and increases the electrical resistance between the electrodes. A voltage pulse of magnitude proportional to the particle volume is thus produced. The resulting series of pulses are electronically amplified, scaled and displayed on a suitable display.

The ratio of the aperture length to the diameter of the aperture play a vital role to achieve optimum performance and to enable the relationship of change in resistance with volume of the cell to hold good it should be 0.75:1.for example the aperture of 100 m diameter the length should be 75 m. to achieve satisfactorily result using coulter principle the average diameter of the particles ranges should be between 2 to 40% of the diameter of the measuring hole. Therefore, the following condition must be met for the measuring range:

$$D/50 \leq d \leq D/2 \ldots\ldots\ldots\ldots \quad (3.2)$$

Where: d = maximum particle size, D = diameter of the measuring aperture.

The lower limit of measurement in the system is governed by the noise sources involved. The noise

Sources include the thermal noise of the detector due to the resistance of the fluid flowing across the orifice and the noise inherent in the electronic circuits. Particles of sizes larger than the diameter of the measuring aperture can only pass through the aperture if their longest dimension is parallel to the axis of the measuring aperture; otherwise they cause the clogging of the aperture. The upper limit of measurement is thus imposed by the increasing size of the particles. When the size of the particle approximates the diameter of the aperture, an amplitude linearity error is produced. Therefore, to count particles of different sizes, the diameter of the measuring aperture must be chosen in such a way as to meet the conditions of measurement. The needed aperture size range to cover measurement size from about 0.5 microns to upwards of 500 microns. (Raghbir Singh Kandpur, 2003)

The figure3.7 below explains the principle of coulter counter.it constructs of glass tube with small aperture in the bottom which immersed into a container that contains particles suspended in a low-concentration electrolyte. . Two electrodes, one inside the aperture tube which called cathode and one outside the tube but inside the container which called anode, are placed and a current path is provided by the electrolyte when an electric field is applied. When the cell suspension is drawn through the orifice, cells will displace their own volume of electrolyte and cause a resistance change, which is converted to a voltage change, and is amplified and displayed. The

cell suspension is drawn through the orifice by means of a mercury manometer. This manometer includes two platinum wire contacts (A and B) set through the glass walls. Contact A will start the count and contact B will stop it when precisely 0.5 ml of the dilution has passed through the orifice tube. Thus, it provides a count of the number of particles in a fixed volume of suspension. The sequence of pulses are generated when resistance increase in at different positions of the cell with respect to the orifice. To count those pulses which fall within certain preset size limits, the threshold facility is required. The threshold is necessary to enable the instrument to ignore any electronic noise, which may be present in the system. The lower threshold sets an overall voltage level, which must be exceeded by a pulse before it can be counted. The upper threshold will not allow pulses to be counted which exceed it preset level. The Coulter counters are usually provided with an oscilloscope monitor to display the pulse information, which has passed through the amplifier, and acts as a visible check on the counting process indicating instantaneously any malfunctions such as a blocked orifice. (Raghbir Singh Kandpur, 2003)

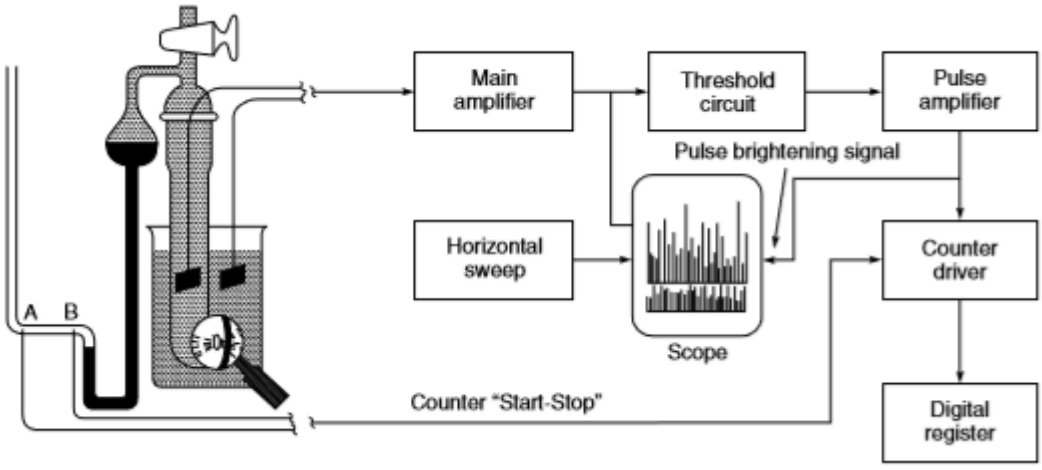


Figure 3.7 Principle of coulter counters

Based on the same principle of detecting change in conductivity in the presence of a cell in the orifice of a measuring tube, there is another cell counting instrument known by the name Picoscale. This instrument does not make use of a mercury manometer for fixing the volume .because the mercury gets dirty as a consequence of

which the contact bordering the volume also dirties the neighboring glass wall which causes uncertainty in counting. (Raghbir Singh Kandpur, 2003)

3. Flow cytometer method

Flow cytometry is one of a technique or method used to count and analyzed the size, shape and properties of individual cells with in heterogeneous population cells.it contain several key components: including the sample fluidic that move the sample in flow cytometer, lasers, optic which gather light, detector to sense the light and computer system to output the data. The blood cell is subjected to fluid dynamic which leads them to flow in a single line. Each cell will pass through a detection device called flow cells which have a laser beam device focused on it and as the cell passes through the laser light path it will scatter light in several directions .the flow cytometer detects light scattered in a forward manner called, forward scatter and the light scattered in sideway manner called, side scatter .the amount of forward scatter from each cell is detected by detector on the far side of cell from the laser this detector called forward laser light(FSL). The forward scatter is proportional to the size of the cell. The detector covert scatter light into a voltage plus which is directly proportional to amount of forward scatter light the computer convert this data into histogram plot with the amount of forward light on the x- axis and a number of cells in a y-axis. The amount of side scatter is detected by detector located perpendicular to the path of the laser beam this detector called side scattered (SS). Side scatter is proportional to the shape and internal complexity of a cell. The flow cytometer converts the detected side into a voltage plus which a directly proportional to amount of side scattered light.by analyzing forward and side scatter data together you can understand cell size, shape and complexity. In addition to analyze a cell's size, shape and complexity the flow cytometry can also detect emitted light from excited fluorescent molecules. But flow cell format has disadvantages which are the more expensive to manufacture and typically fixed to one Chanel width while the aperture format offer wide verifying of aperture size. (DP Lokwani, 2013)

3.4 Types of automatic blood cell counter

We can divide the automatic blood cell counter to two main types of blood cell counter based on the differentiation of WBCs which are three-part and five-part blood cell counter. Impedance technology can deliver a three part WBC differential, where cells are grouped into three sizes: lymphocytes, mid-range cells and granulocytes but it does not allow for the differentiation of the granulocyte sub-type such as nucleated red blood cells (NRBCs), PLT clumps, giant PLTs or un-lysed red cells, cannot be separated and may interfere with normal cell populations. While Optical flow cytometry provides several advantages over traditional impedance methods.it provide more information about cellular characteristics such as size, internal complexity, nuclear globularity segmentation and cytoplasmic granularity. The use of optical technology has led to the ability to provide five-part WBC differentiation where cells are grouped into three sizes neutrophil, eosinophil and basophil granulocytes, lymphocytes and monocytes.

In addition to above two types some analyzer my report six-part WBCs differential or seven-pat WBCs differential by adding fluorescence dye to enhance optical flow cytometer technology .integrated with multiple angle optical light scatter method to further improve the WBCs differential subtype clarification. A sixth category designed large unstained cells larger than normal and lack the peroxides activity this includes atypical lymphocytes and various other abnormal cells.as for seventh-part differential includes five-part plus large immature cells (composed of blast and immature granulocyte) and atypical lymphocytes (including blast cells).(DP Lokwani,2013)

3.5 Automatic instrument for complete blood count

Since the end of the nineteenth century, prototypes of automated blood meters were developed for the first time and throughout the twentieth century and with the rapid development, many manufacturers have developed and manufactured automated blood cells counter such as Bayer coulter, Beckman coulter, Abbott, sysmex, mindray and others manufacturer of hematology analyzer. These instruments performed measurements using one of major classes for counting and characterizing blood cells for disease detection and monitoring. One type is based on electric re-

sistance of a solution when a blood pass through aperture and other type utilize deflection of light beam caused by passing of blood or a combination of both optical and impedance-based methods. We review one of these tools, which are one of the tools of hematological analysis, and represent the apparatus that was researched. (Webster, 1998)

3.5.1 **Mindray**

The sample that will be analyzed is blood sample that has been anti-coagulated with ethylenediaminetetraacetic acid (EDTA) which are substances interfere with the normal clot forming mechanism of blood to keep the formed elements from clamping together by removing calcium from blood which would prevent them from being counted accurately.(Webster,1998).

This analyzer used the Coulter method for determining the WBC, RBC, and PLT data and the colorimetric method for determining the HGB.it can process two types of blood samples – whole blood samples and pre diluted blood samples. In case a whole blood sample, you can simply present the sample to the sample probe and press the aspirate key to aspirate 13µL of the sample into the analyzer as for analyze a capillary blood sample, you should first manually dilute the sample (20 µL of capillary sample needs to be diluted by 0.7mL of diluent) and then present the pre-diluted sample to the sample probe and press the aspirate key to aspirate 0.3ml of the sample into the analyzer.

3.5.1.1 **Whole blood mode Counting cycle**

When analyzing a whole blood the initial step is aspirates 13µL of the blood sample into probe. The instrument read HGB blank then rinses, drains WBC bath and dispense diluent to prefill the WBC bath. The probe move to WBC bath and dispense the sample 13ul and diluent into the WBC bath, making 1:269 dilution. . The RBC bath rinses, drains and dispenses diluent into the RBC bath to prefill it. The 50ul syringe aspirates 15.6ul of the 1:269 dilutions into the probe for the RBC/PLT dilution. The 2.5ml syringe aspirates lyse. The lysing agent cause the cell membrane of the RBC rupture and release hemoglobin into solution and WBC dose not rupture by this solution. then The 2.5ml syringe sends 0.5ml lyse reagent to the WBC bath for a final 1:308 dilution, while the 10ml and 50ul syringes dispense 15.6ul of the 1:269 di-

lution and additional diluent into RBC bath for a final RBC/PLT dilution of 1:44833. Mixing bubbles enter the baths to mix the bath contents. The vacuum chamber drains and both dilutions (WBC and RBC/PLT) are drawn through the apertures via regulated vacuum. The instrument counts 500ul dilution for WBC and counts 300ul dilution for RBC and PLT. After counting finishes, the flow ends. The system takes an HGB sample reading and analyzes the data while the WBC and RBC baths drains and rinses. Finally the system zaps the apertures and the probe moves to the aspirating position. The system displays results on the screen and be ready for the next sample. The figure (3.8) bellow explains these steps. (Shenzhen Mindray, 2005)

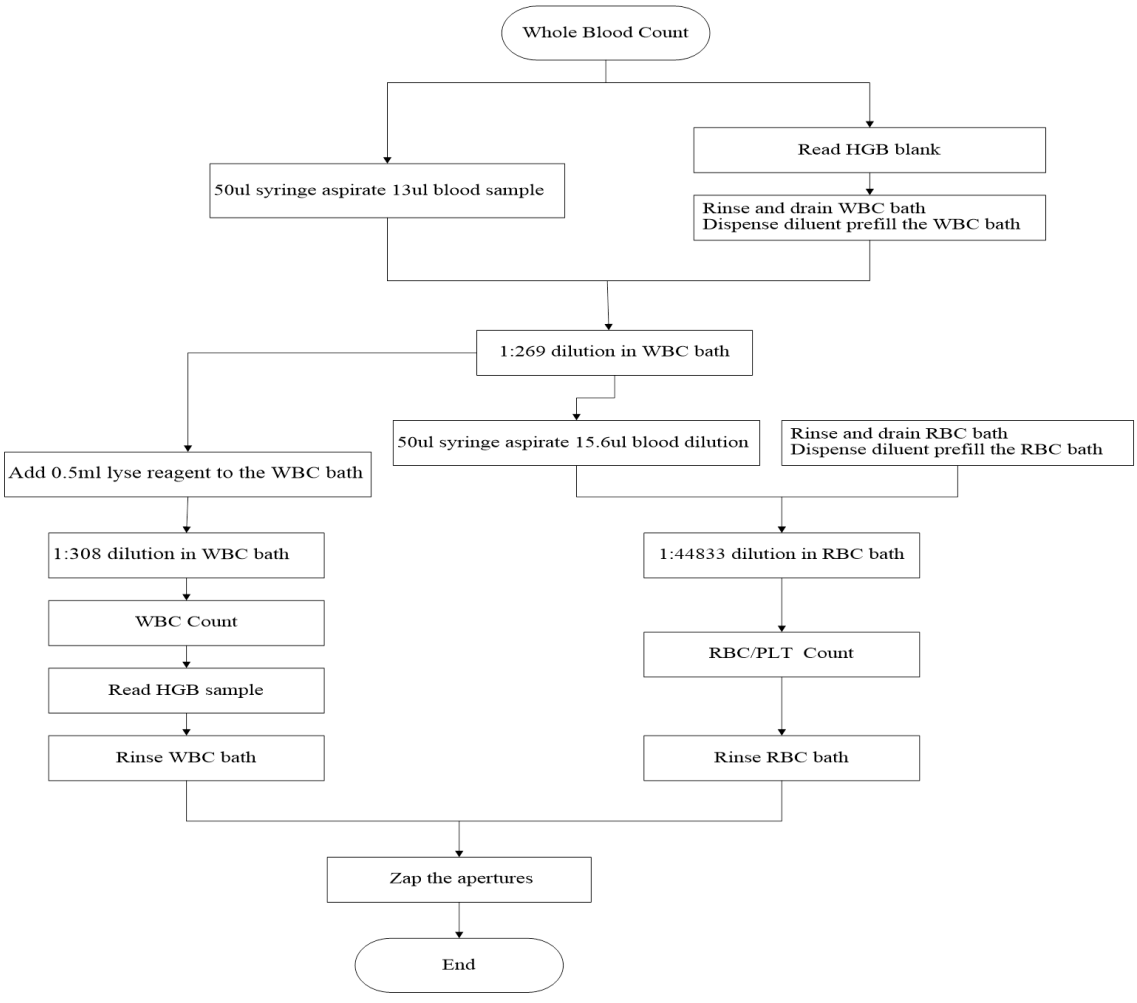


Figure 3.8 Whole blood counting cycle

3.5.1.2 **Pre diluted blood mode Counting cycle**

When analyzing a pre-diluted sample, you should first collect 20μL of capillary sample and dispense 0.7mL of diluent from this analyzer to predilute it. Then the analyzer aspirates 0.3mL of the pre-diluted sample for further dilution, as Figure3.9 shows. (Shenzhen Mindray, 2005)

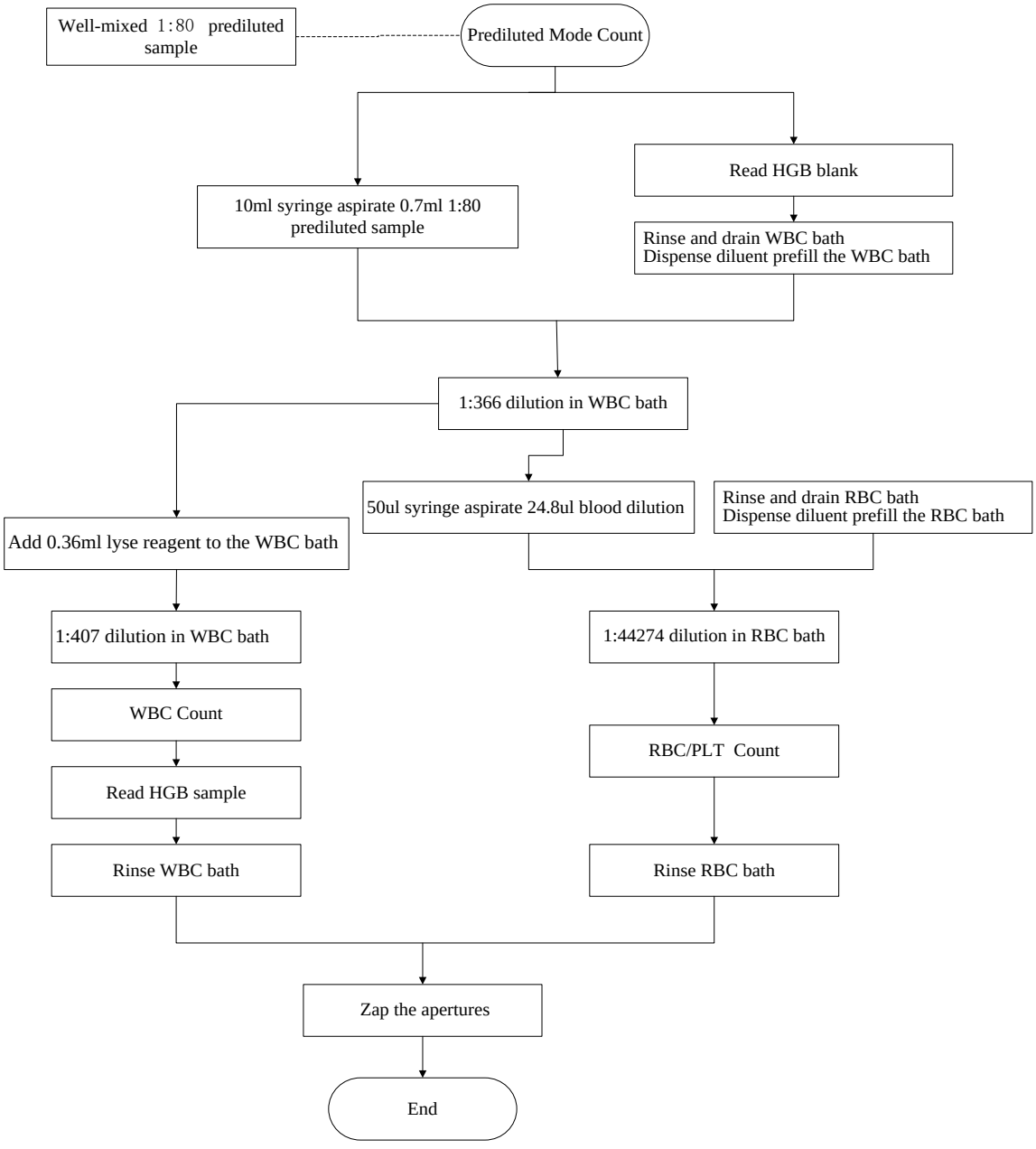


Figure 3.9 Predilute blood sample counting cycle

3.5.2 **WBC/HGB Measurement**

The method for obtaining an accurate cell number is by the precise size of the diluted sample passing through the aperture during the counting part of the analysis cycle is known .which known as volumetric unit that used to control the count cycle and to ensure an accurate sample size analysis in this analyzer. The metering unit controlling the WBC count cycle consists of a metering tube with two optical sensors mounted on it. This tube ensures that a precise amount of diluted sample is measured during each count cycle. The exact amount is determined by the distance between the two optical sensors. The rinse is used to create a meniscus in the metering tube. The count cycle starts when the meniscus reaches the upper sensor and stops when the meniscus reaches the lower sensor. The amount of time required for the meniscus to travel from the upper sensor to the lower sensor is called the WBC Count Time and is measured in seconds. At the end of the count cycle, the measured count time is compared to the pre-defined reference count time. If the former is less than or greater than the latter by 2 seconds or more, the analyzer will report it as an error. (Shenzhen Mindray, 2005)

WBCs are counted and sized by the Coulter method principle. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in a conductive diluent as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated signals the number of particles that passed through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each pulse is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the WBC threshold, it is counted as a WBC. (Shenzhen Mindray, 2005)

As for HGB, it is determined by the colorimetric method. The WBC/HGB dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is measurable at 525 nm. An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then is measured by a pho-

to-sensor that is mounted on the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading. The HGB is calculated per the following equation and expressed in g/L.

HGB (g/L) = Constant ×Log 10 (Blank Photocurrent/Sample Photocurrent)….(3.3)

3.5.3 **RBC/PLT Measurement**

To obtain an accurate cell number in the exact size of the diluted sample passing through the aperture during the counting part of the analysis cycle is known .it known as volumetric unit which is the same method that used in WBC / HGB that explained earlier.

The RBCs/PLTs are counted and sized by the Coulter method. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in a conductive diluent as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated signals the number of particles that passed through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each pulse is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the RBC/PLT lower threshold, it is counted as a RBC/PLT. (Shenzhen Mindray, 2005)

3.5.4 **Complete blood count parameters**

There automatic hematology analyzer can give out 19 parameters and 3 histograms or more depend on the type of the instrument. It can be divided into 3 results coming from measurement, results derived from the histogram and results calculated by formula. The measurement parameters are WBC, RBC, PLT and Hg which has been explained the way of measurement previously.

The WBC (109/ L) is the number of leukocytes measured directly by counting the white blood cells passing through the aperture.it should ensure that nucleated red blood cells (NRBCs) or other conditions are not falsely affecting the WBC

which do not react with the lyse and can be mistaken by the analyzer for white cells. Modern instruments are able to identify NRBCs and subtract them from the WBC value, thus ensuring that the WBC represents only WBCs. In instances when an instrument-corrected WBC value is not available, a manual correction must be applied using the microscope to be sure and correct the system-generated result by the following formula.

$$WBC'' = WBC*(100/(100+NRBC)) \quad \ldots\ldots\ldots\ldots\ldots (3.4)$$

Where: WBC" the corrected white cell number, WBC is represents the system-generated white cell number and NRBC the number of NRBCs counted in 100 white cells.

Based on the WBC histogram and by helping of the diluent and lyse the analyzer differentiate the WBC into three sub-populations which are lymphocytes, mid-sized cells(monocytes, basophils and eosinophil) and granulocytes.it calculates Lymph%, Mid% and Gran% as follows and express the results in percent.

$$Lymph\% = (PL/(PL+PM+PG))*100\ldots\ldots\ldots\ldots\ldots (3.5)$$

$$Mid\% = (PM/(PL+PM+PG))*100\ldots\ldots\ldots\ldots\ldots (3.6)$$

$$Gran\% = (PG/(PL+PM+PG))*100\ldots\ldots\ldots\ldots\ldots (3.7)$$

Where PL are particles in the lymphocyte region (L/109), PM are particles in the mid-size region (L/109) and PG are particles in the granulocyte region (L/109).Then it can calculate the Lymph# , Mid# and Gran# by the following equations and express them in L/109.

$$Lymph\# = (Lymph\%*WBC)/100\ldots\ldots\ldots\ldots\ldots (3.8)$$

$$Mid\# = (Mid\%*WBC)/100\ldots\ldots\ldots\ldots\ldots\ldots (3.9)$$

$$Gran\# = (Gran\%*WBC)/100\ldots\ldots\ldots\ldots\ldots (3.10)$$

Beside the parameters it has WBC histogram whose x-coordinate represents the cell volume (fL) and y-coordinate represents the number of the cells in addition to two other histograms for RBC and PLT. (Doig, Thompson, 2017; Shenzhen Mindray, 2005).

As for the RBC (1012/L) is the number of erythrocytes measured directly by counting the erythrocytes passing through the aperture. In some types of hematology instrument and based on the RBC histogram the mean cell volume (MCV) or the average volume of individual red cells can be measured directly and expresses the re-

sult in fL while derive hematocrit (HTC) or vice versa measured HTC and calculate MCV but the technique of the hematology analyzer under the study is to measure MCV. Then it can calculate HCT (%), Mean Corpuscular Hemoglobin (MCH (pg)) which is a measurement of the average weight of hemoglobin in individual red cells and Mean Corpuscular hemoglobin Concentration (MCHC) which is the average concentration of hemoglobin in grams in a deciliter of red cells (g /L) as follows:(Abdul Hamid,2012)

HCT (%) = (MCV*RBC)/10…….……..…… (3.11)

MCH (pg) = (HGB (g/dl)/RBC (L))*10……..…… (3.12)

MCHC (g/L) = (HGB g/dl)//HCT (%)) *100……..…… (3.13)

In addition to that it has RDW is the mathematically expression of variation in size of RBC (or anisocytosis) which is calculated by the cell counters. The RDW is the coefficient of variation of the normally Gaussian curves shaped RBC volume distribution histogram. The RDW is determined by dividing the standard deviation of the mean corpuscular volume (MCV) by MCV and multiplying by 100 to convert to a percentage value. Thus the RDW is a quantitative measure of the size variation of circulating RBCs. The normal value for RDW is 12-15 % figure below explains RDW. (Abdul Hamid, 2012)

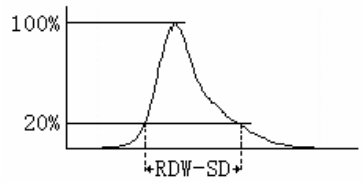


Figure 3.10 Red blood cell distribution widths (RDW)

With regard to PLT (109/L) is measured directly by counting the platelets passing through them aperture and from the PLT histogram can measure Platelet distribution width (PDW) is the geometric standard deviation (GSD) of the platelet size distribution which is derived from the platelet histogram data and is reported as 10(GSD) also can calculates the mean platelet volume (MPV) in fl from histogram this only for the instrument under the study other instrument may have differ method. Then it can calculate PCT as follows: (Abdul Hamid, 2012)

PCT= (PLT*MPV)/10000……..…… (3.14)

CHAPTER FOUR

METHODOLOGY

METHODOLOGY

The methodology that is follow in the research is as following:

4.1 Study design

This research is a cross sectional study design which assesses the effect of some parameters on the auto hematology analyzer instrument.

4.2 Study Area

This study was carried out in different Medical Laboratories that are using auto-hematology analyzer in hospitals, clinics, health and centers dispensaries located in Khartoum state Sudan.

4.3 Study type

A cross sectional study was conducted using questionnaire by Face-to-Face interview to supervisor of the Laboratory selected and agreed to do assessment to it instrument.

Cross-sectional studies can be defined as studies that are carried out at one time point or over a short period or a study of a single, natural sample with concurrent measurement of a variety of characteristics They are usually conducted to estimate the prevalence of the outcome of interest for a given population, commonly for the purposes of public health planning. Data can also be collected on individual characteristics, including exposure to risk factors, alongside information about the outcome. In this way cross sectional studies provide a snapshot of the outcome and the characteristics associated with it at a specific point in time. (Johan M. Lachin, 2000)

4.4 Study Population:

Study population is the auto hematology analyzer instrument .it was included one brand of different batch because it was available and use in most medical laboratory in chosen hospital and clinics in Khartoum state.

4.5 **Sampling Technique and Sample Size**

In this research was used simple random sampling technique which means every medical laboratory use auto hematology analyzer has an equal chance of getting selected to be a part of sample for the sample size of 30 instruments was investigated in different laboratories. The Sample size for auto hematology analyzer was calculated as follows:

$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2} \qquad \text{……..(4.1)}$$

n=sample size.

z =Confidence Level (level of significance) (95% or 1.96).

ε= margin of error of proportional18%.

$\hat{p}$ = population proportion. If population proportion equal 50%

$$\mathbf{n} = \frac{(1.96)(1.96)(0.5)(0.5)}{(0.18)(0.18)} = 29.6413 = 30$$

4.6 **Population criteria:**

4.6.1 **Inclusion criteria:**

1. All Medical Laboratories offering complete blood count test using mindray hematology analyze.
2. Auto hematology analyzer instrument use principle of electrical impedance method for counting cell, colorimetric for measuring HB and 3 differentials to differentiate white blood cell (WBC) in addition to full automatic sampling, rinsing, diluting and un clogging were included in the study.
3. The medical laboratory technologist or specialist who had used auto hematology analyzer instrument, worked at the Laboratory at least for two year and accepted to be interviewed or filled questionnaire during the study became eligible to be included in the study

.

4.6.2 **Exclusion criteria**

1. The Medical Laboratories offering complete blood count test using other manufacture instruments
2. Auto hematology analyzer instrument use 5-part differentiation or Tri-angle laser scatter, chemical dye and Flow cytometer technology.
3. All CBC Parameters not measured except HTC.
4. The medical laboratory technologist who had worked in auto hematology for less than two year were excluded from the study; this was because the questionnaire were designed in such a way that respondents need to have at least an experience on her job. It was assumed that two year would be enough time for a medical laboratory technologist or specialist to answer the questions in the questionnaire in addition to medical laboratory technologies who were not able to respond to the questionnaire were not included in the study

4.7 **Criteria of selected reference instrument**

A reference instrument was selected to use as reference to other instruments under study The instrument was chosen for two reasons .the main reason is the availability of the instrument and other reason have same method of measurement of instruments under study and the same brand. It performance was evaluated and compared with two instruments of other brand have the same methods of measurement.

4.8 **Sampling procedure**

All Medical Laboratories in the state were stratified into three (3) categories (hospital lab, health center lab and dispensary lab) as per level of health facilities service delivery. There after a stratified randomly selection were done from each category**.**

4.8.1 **Selection of laboratory technologist /specialist**

The study conveniently selected the laboratory technologist /specialist who participated in the study .From each laboratory selected a senior of lab were asked to be interviewed to fill the questionnaire .A 30 laboratory technologist /specialist were interviewed.

4.8.2 Selection of auto hematology analyzer

A quick survey was done using phone to know most used hematology analyzer instrument in the most medical laboratories in the Khartoum state and then was determine the specific brand of instrument for the study which is using in the majority laboratories.

4.9 Data collection methods

Data was collected using questionnaire in addition to instrument checkup and evaluated study. The questionnaire was produced in English and was used for interviewing to obtain information about user, environment where the instrument use, instrument and technical knowledge.

As for instrument checkup and study was done to obtain data about current situation of instrument in the day of investigation. It was divided into two sections. First section was focused on primary information of instrument, checkup the instrument, measure room temperature and humidity where the instrument was using. Second section was concentrated on back ground checkup, precision, accuracy and carryover of some parameters as follow:

I. Instrument was checked by entering to menu and select service tab then was done the system status check, valve test and system test.

II. Room temperature and humidity were measured using temperature and humidity meter and 5 reading were taken every 15 minutes and the average of it was calculated.(see appendix A)

III. Back ground checkup was done enter “0” into the “ID” box Compare the result if it is in permissible range or not. (see appendix B) (Shenzhen Mindray, 2005).

IV. In the precision, accuracy and carryover study firstly was selected an auto hematology analyzer to use as reference instrument after doing study on it and it result was compared with 2 instruments of different manufacture used the same principle and method to make sure that it is accurate and precise and then blood sample was read on it firstly and was used the result that measured on it as reference value to other instruments under study.

V. To measure precision of instrument measurement the reference blood sample was measured 11 times consequently then measured the closeness of blood sample measurement to each other it should give narrow different in value less or equal than specific value of coefficient of variation (CV) as in appendix C.(Shenzhen Mindray, 2005; product guide, 2019)

VI. In carry over test any blood sample was run 2 times respectively follow by running blank 3 times respectively then was used special formula to calculate carry over and compare it result if it is less than or equal permissible range or not.(see appendix D) (Shenzhen Mindray, 2005). HCT should be less than or equal 0.5% because it is a calculated parameter whose value depends on the RBC when calculated.

VII. The accuracy test which refers to closeness of the measurements to a specific value it was done by measuring reference blood sample in instrument under study and subtract it result from acceptable measure. value of subtraction result should be plus or minus specific value for the instrument to be accurate in it measure .The permissible value are 1,0.24,0.6,2,40 for WBC,RBC,HGB,HCT and PLT sequentially.(see appendix E) (Hematology Controls and Calibrators, 2020)

4.10 **Blood Sample collection**

It used blood collected for apheresis laboratory for testing of CBC for donors that came to donate by plasma or platelet. The blood was drawn into polyethylene terephthalate plastic tubes containing K2-EDTA by experienced laboratory technologist where the CBC was performed on reference instrument .then the same sample was analyzed within 2 h to 6h in other instrument in other laboratory and the results were recorded in the questionnaire form. Specimen was transported between laboratories at room temperature. The study was consisted of 30 auto hematology analyzer in 30 different laboratories .It used 30 blood samples one sample against each instrument.

4.11 Questionnaire

Primary data were collected by using structured questionnaire with open, closed ended questions; instrument checkup and study which were written in English. Questions were asked on staff competency information, user training, operation, instrument and technical knowledge (standard check, preventive maintenance, calibration) and environment. Then information on performance related factors was collected through structured study whereby checkup the device and room temperature, background, precision, carry over and accuracy.

The in house survey questions were evaluated by statistical judge from Sudan University of science and technology statistic faculty. (See appendix F and G).

4.12 Data Management

The process of editing data was done during and after data collection.

4.13 Study variables

4.13.1 Dependent Variable

It is called dependent because it depends on the independent variables. Which are precision, accuracy and reliability of WBC, RBC, HG, HTC and PLT using auto hematology analyzer which was used measured the performance of instrument.

4.13.2 Independent Variables

1. Age of respondent (in complete year).
2. Sex of respondent (male, female).
3. Qualification of respondent (Diploma, Bachelor's degree, Master's degree, Doctoral degree).
4. Years of Experience (in complete year).
5. Staff and supervisor training (Yes, No).
6. Operation (Yes, No).
7. Standard check (Yes, No).
8. Preventive maintenance (Yes, No).
9. Q.C (Yes, No).
10. Calibration (Yes, No).
11. Instrument environment (Yes, No).

Staff competency parameter which are age, sex, qualification and years of experience are necessary to define skills, ability to perform and knowledge.as for others variables which are staff and supervisor training ,operation, Standard check ,preventive maintenance ,Q.C, calibration and instrument environment are impact on instrument efficiency ,accurately and keeping it on good operating condition,

4.14 **Data processing and Analysis:**

After completion of the field work in all the selected areas, questionnaires and study of the same laboratory were given same serial numbers before data entry. After completion of the field work in all the selected areas, questionnaires and studies of the same laboratory were given same serial numbers and calculation process of some parameters in the studies were done firstly before data entry.

Data were entered and analyzed by using SPSS version 26.0 where by frequency tables were run and percent were calculated. Also the researcher use correlation analysis to measures the association between two variables (independent and dependent variables) and the strength of their relationship.

Auto hematology analyzer performance was measured by using four job performance indicators which are availability, productivity, competence and responsiveness.

4.15 **Limitation of the study**

1. This study looked at a specific type of auto hematology analyzer in Khartoum state which raises potential for sampling bias. However these limitations could be considered as an opportunity for future research on the effect of some parameters on the performance of auto hematology analyzer which may include auto hematology analyzer instrument used in all laboratories. It may also include both public and private health facility.

2. In this research was used sample measured on selected reference hematology analyzer instead of using commercial whole-blood products and compare with reference range of control sample in assessment of accuracy. This may effect on the reliability of accuracy test. Even though it requested control sample from the company but it apologized for my request due to little quan-

tity required compared with the cost of deportation and the long procedures of government which was lead to receive it expired. It can avoid all this limitation if have locally manufactured standard control sample.

CHAPTER FIVE

RESULT AND DISCUSION

RESULTS & DISSCUSION

5.1 Results

This chapter presents the results of the study. It starts by describing the staff-competency characteristics, and then followed by substantive findings of the study.

5.1.1 Staff competency characteristic of the study population

5.1.1.1 Staff competency characteristic of the interview Respondents

Table 5.1 staff competency characteristic of the interview Respondents

Characteristic		frequency	Percent (%)
Gender	Male	14	46.7%
	Female	16	53.3%
Age grouped in years	≤ 35 years	21	70%
	>35 years	9	30%
Educational level	Diploma	1	3.3%
	bachelor degree	14	46.7%
	master degree	15	50.0%
	doctoral degree	0	0.0%
Years' experience	< 1year	0	0.0%
	1-5years	10	33.3%
	6-10years	8	26.7%
	11 years and above	12	40.0%
level of health facility	Hospital	15	50.0%
	health center	1	3.3%
	Dispensary	5	16.7%
	special lab	9	30.0%

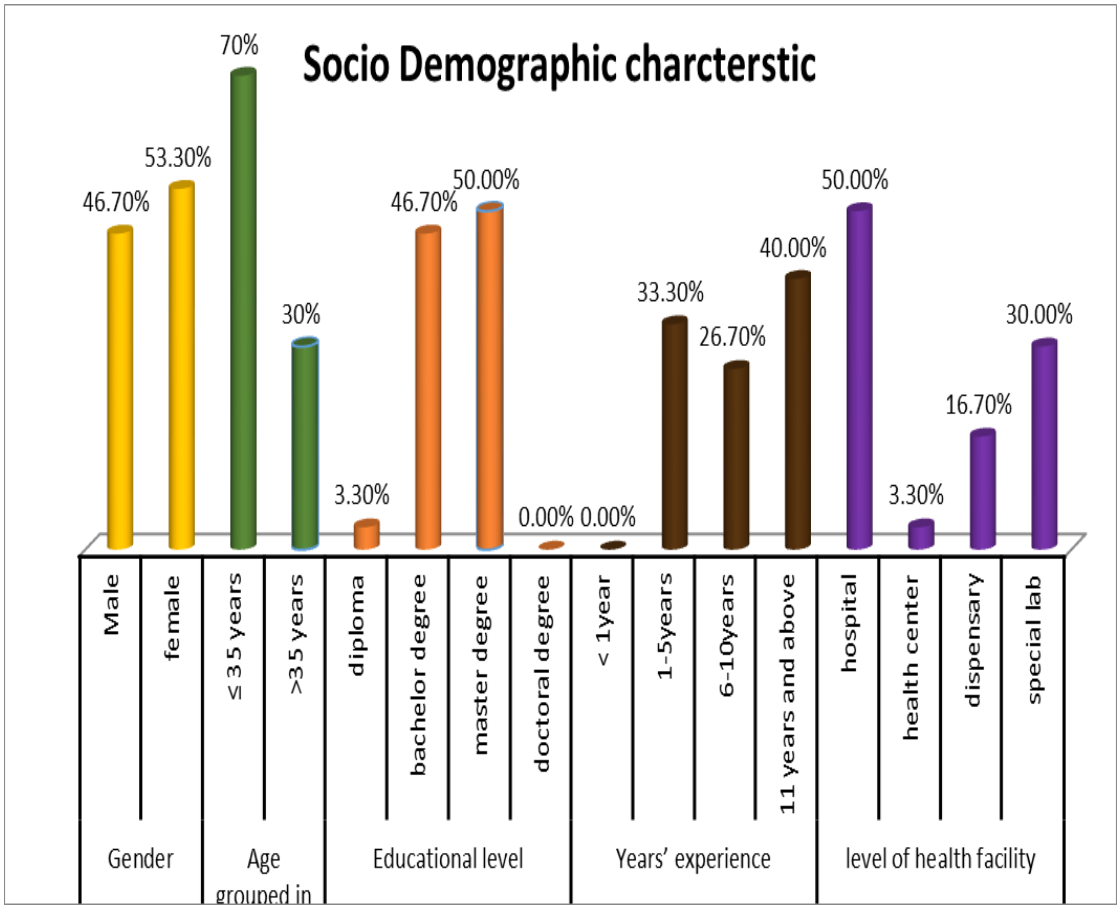


Figure 5.1 staff competency characteristic of the interview Respondent

A total of 30 laboratory specialist working in the medical laboratory have auto hematology analyzer in 30 health facilities (15 hospital, 1 health center, 5 dispensaries and 9 special lab) were interviewed.

The table 5.1 as figure 5.1 shows that the highest respondent percent of total sample were female by 16 (53.3%) while male were 14 (46.7%) of the total sample. About age we noted that majority age were less than 35 years which represent the highest percentage (70 %). Furthermore the Table 5.1 shows that among respondents 50 percent had master degree and no one has doctoral degree. As for years of experience 40 % of respondents had the experience of 11 years and above while other respondents those who had the experience of 6-10 years were 26.7 % and 1-5years were 33.3 %.

5.1.1.2 **Characteristic of auto hematology analyzer:**

Table 5.2 Shows the summary of auto hematology analyzer characteristics

Characteristic		frequency	Percent (%)
Level of health facility	Hospital	15	50.0%
	health center	1	3.3%
	Dispensary	5	16.7%
	special lab	9	30.0%
Insulation date	2010	1	3.3%
	2013	7	23.3%
	2014	4	13.3%
	2015	6	20.0%
	2016	5	16.7%
	2017	5	16.7%
	2019	2	6.7%
Instrument duration of use(by years)	1	2	6.7%
	2	5	16.7%
	3	5	16.7%
	4	6	20.0%
	5	4	13.3%
	6	7	23.3%
	10	1	3.3%
Manufacturer data	2008	2	6.7%
	2010	2	6.7%
	2012	5	16.7%
	2013	4	13.3%
	2014	7	23.3%
	2015	4	13.3%
	2016	4	13.3%
	2017	1	3.3%
	2018	1	3.3%
	2019	0	0.0%

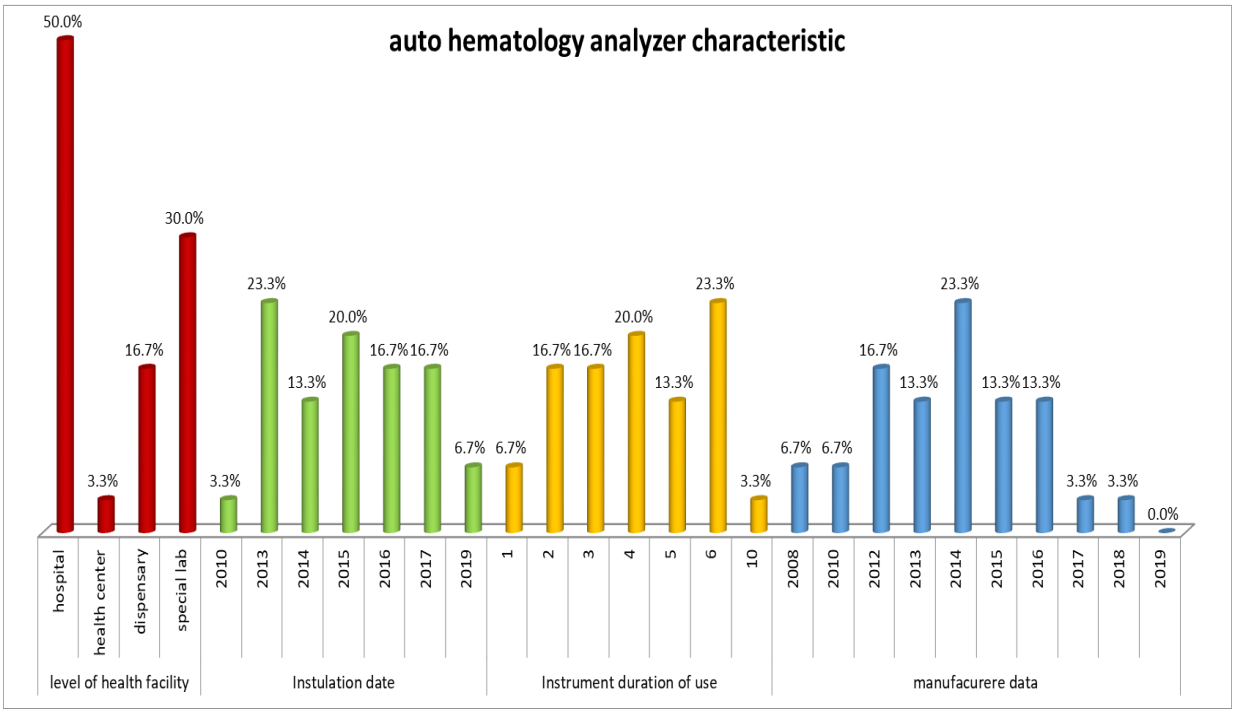


Figure 5.2 Auto hematology analyzer characteristics

A total of 30 auto hematology analyzers were evaluated in 30 medical laboratories (15 hospital, 1 health center, 5 dispensaries and 9 special labs) were evaluated. Table 5.2 and figure 5.2 show that the highest duration period of instruments were 6 years which represent 7(23.3%) installed in 2013while other durations were 4 years 6(20%) installed 2014, 3 years 5(16.7%) installed 2016, 2 years (16.7) installed 2017, one year 2(6.7) installed 2019and 10years 1(3.3%) installed 2010.

5.1.2 **The reliability and validity analysis of auto hematology analyzer performance Concepts**

Validity and reliability analysis were done to so as to check if the items explain the auto hematology analyzer performance measures (accuracy, precision and reliability) in the chosen medical laboratory. The table 5.3 shows the Cronbach's Alpha reliability coefficient. The first column shows the number of questions or variables that were entered in the calculation of the alpha coefficient and the second column gives us the value of the reliability coefficient and is equal 0.715, and therefore the questionnaire has high constancy reliability and the validity coefficient 0.846.

Table 5.3 Alpha coefficient to measure the reliability of the study tool

Cronbach's Alpha	N of Items
0.715	44

5.1.3 **User training assessment**

Table 5.4 User training

User training variables		Frequency	Percent
Adequate training in using instrument	Yes	26	86.7
	No	4	13.3
Instrument use only by trained staff	Yes	26	86.7
	No	4	13.3
Writing SOPS	Yes	10	33.3
	No	20	66.7
Well trained engineer in maintenance	Yes	19	63.3
	No	11	36.7
Trained in QC	Yes	4	13.3
	No	26	86.7

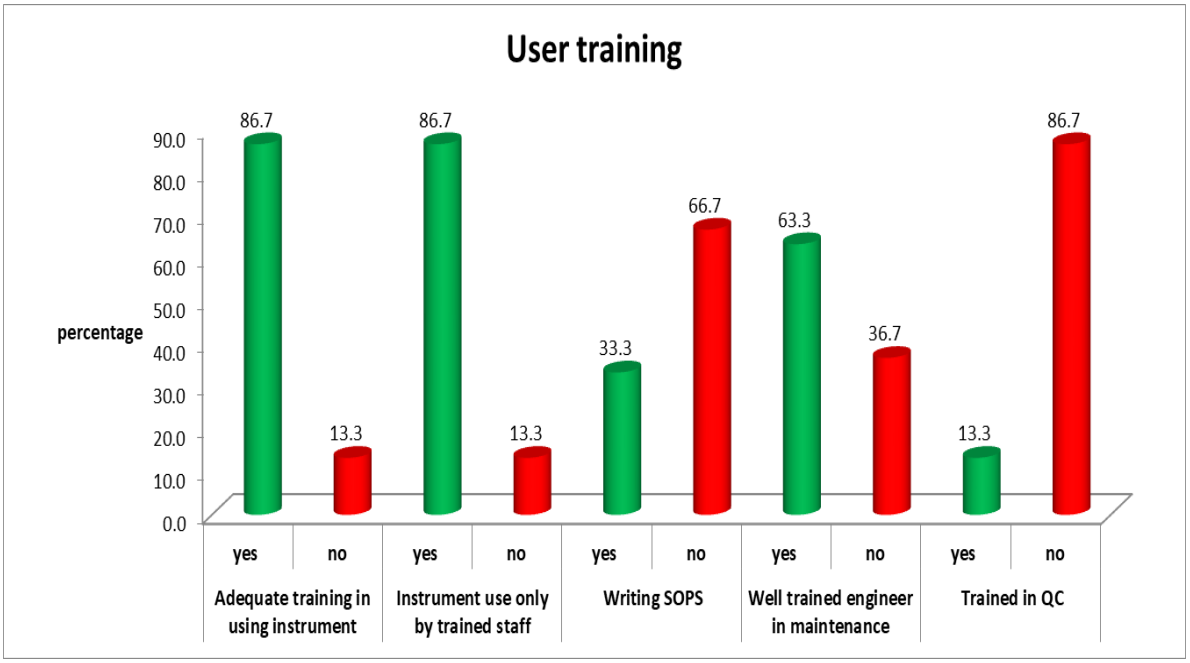


Figure 5.3 User training

Table 5.4 as figure 5.3 show that the percent of user that had trained was 26(86.7%) and the instrument used only by trained staff 26(86.7%) while the percent

of use that have trained in Q.C was 4(13.3%).As for engineer 19(63.3%) was trained and 10(33.3%) have writing SOPS.

5.1.4 **User training associate with health level facility**

Table 5.5 User training by health level facility

User training variables		level of health facility							
		Hospital		health center		Dispensary		special lab	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1.	Yes	13	43.3	1	3.3	5	16.7	7	23.3
	No	2	6.7	0	0.0	0	0.0	2	6.7
2.	Yes	13	43.3	1	3.3	5	16.7	7	23.3
	No	2	6.7	0	0.0	0	0.0	2	6.7
3.	Yes	6	20.0	0	0.0	2	6.7	2	6.7
	No	9	30.0	1	3.3	3	10.0	7	23.3
4.	Yes	6	20.0	1	3.3	5	16.7	7	23.3
	No	9	30.0	0	0.0	0	0.0	2	6.7
5.	Yes	2	6.7	0	0.0	2	6.7	0	0.0
	No	13	43.3	1	3.3	3	10.0	9	30.0
(1, 2,3,4 and 5 are user training variable code .(1)Adequate training in using instrument,(2)Instrument use only by trained staff,(3)Writing SOPS,(4)Well trained engineer in maintenance and (5)Trained in QC.									

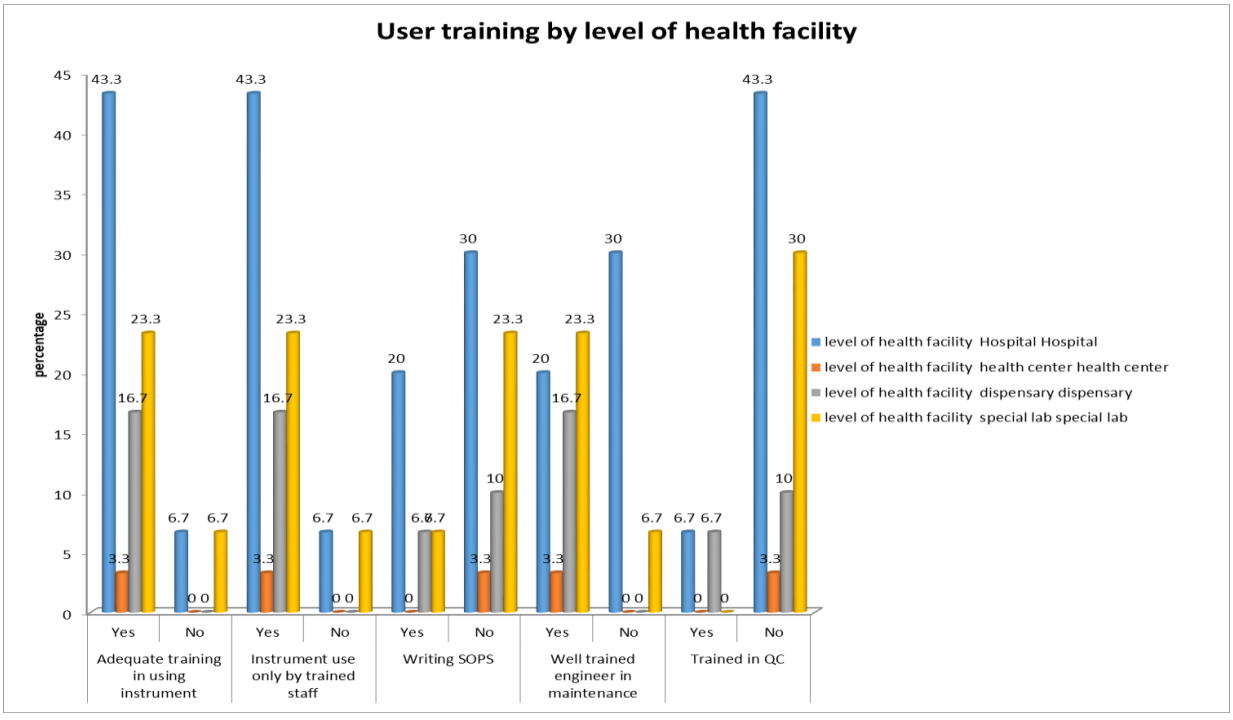


Figure 5.4 User training by health level facility

From table 5.5 and figure 5.4 shows the summary of user training among health level facility .Majority of the trained users 13 (43.3%) from hospitals and 13 (43.3%) the maximum percent of instrument used only by trained staff also from hospitals among users trained in Q.C 2(6.7%) from hospitals and2(6.7%) from Dispensaries have equal percent . Additionally, the table shows that hospitals have high percent between the health level facility that have written SOPS were 6(20%).As for maximum percent of trained engineer was7 (23.3) in special lab.

5.1.5 **Auto hematology analyzer Operational characteristics**

Table 5.6 Auto hematology analyzer Operational characteristics

Operation Variable	study variable		Frequency	Percent
1	Used accordance to manufacturer	Yes	30	100.0
		No	0	0
2	Checking tubes and power connection before using	Yes	17	56.7
		No	13	43.3
3	Doing prime for new reagent	Yes	20	66.7
		No	10	33.3
4	Background check up after install new reagent	Yes	17	56.7
		No	13	43.3
5	Only used manufacture reagent	Yes	30	100
		No	0	0
6	Set reference range depend on patient	Yes	1	3.3
		No	29	96.7
7	Set range depend on Sudanese study	Yes	6	20.0
		No	24	80.0
8	Make sure status area ready and selected mode	Yes	28	93.3
		No	2	6.7
9	Mixing sample before measurement	Yes	29	96.7
		No	1	3.3
10	Action procedure when sample measurement greater or lower than reference range	Repeat in other instrument or by dilution mode	21	70.0
		Manual by microscope blood film	9	30.0

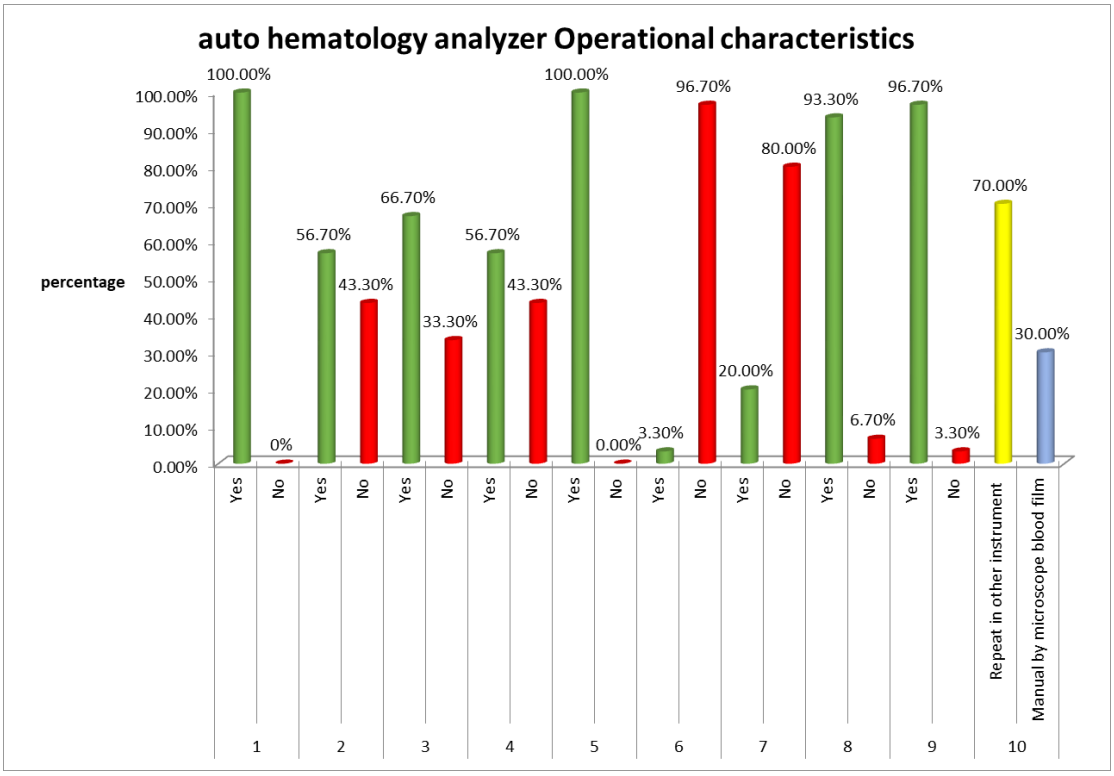


Figure 5.5 Auto hematology analyzer Operational characteristics

The result from table 5.6 and figure 5.5 show 30(100%) of auto hematology analyzer user use accordance to manufacturer instruction, ,30(100%) use only manufacturer reagent ,17(56%) inspect instrument before turn on, 20(66.7%) do prime when install new reagent,17(56.7%) do back ground check up after change reagent,29(96.7%) use general set up(do not selected depend on patient type or age) ,24(80%) use references range set up by manufacturer ,28(93.3) run sample after sure that there is no error massage in status area ,29(96.7%) mixing sample before aspirate it ,21(70%) repeat sample in other instrument when doubt about the result and 9(21%) return to take more information about patient case and it prefer to do peripheral blood film.

5.1.6 **Instrument and technical knowledge**

5.1.6.1 **Standard check**

Table 5.7 Review auto hematology analyzer Standard check

Standard check variable		Frequency	Percent (%)
Schedule Standard check	Yes	4	13.3
	No	26	86.7
Daily background checkup	Yes	20	66.7
	No	10	33.3
Clean sample valve weakly	Yes	3	10.0
	No	27	90.0
Clean WBC and RBC transducer in need	Yes	16	53.3
	No	14	46.7
Use other cleanser	Cholorex	2	6.7
	Not use any other cleaner	28	93.3
Monthly waste chamber clean	Yes	4	13.3
	No	26	86.7

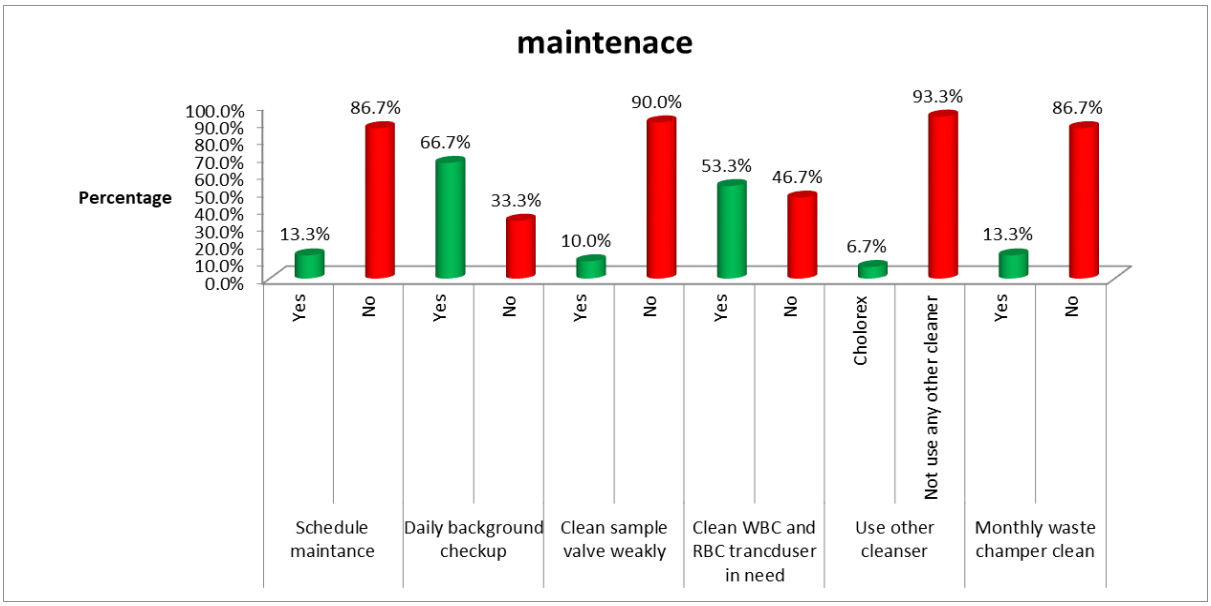


Figure 5.6 Review auto hematology analyzer Standard check

Table 5.7 as well as figure 5.6 shows that 26(86.7%) of medical laboratory do not have schedule Standard check , 20(66.7%) do back ground check up ly ,27(90%) do not do clean sample valve weekly,16(53.3%) do clean WBC and RBC transducer aperture and 28(93.3%) only use manufacturer cleaner when clean

WBC and RBC transducer in addition to that 26(86.7) do not do monthly waste chamber clean .

5.1.6.2 **Preventive maintenance**

Table 5.8 The preventive maintenance program for auto hematology analyzer instruments in the surveyed medical laboratory

Preventive maintenance variable		Frequency	Percent %
Shut down use E_Z cleaner	Yes	28	93.3
	No	2	6.7
Regularly do probe cleanser	Yes	21	70.0
	No	9	30.0
Every month do probe localize	Yes	1	3.3
	No	29	96.7
Clean bath	Yes	8	26.7
	No	22	73.3
Clean wipe block	Yes	4	13.3
	No	26	86.7
Do system test	Yes	11	36.7
	No	19	63.3
Flush to prevent clogging	Yes	25	83.3
	No	5	16.7
Prepare to ship for repacking or movement	Yes	8	26.7
	No	22	73.3

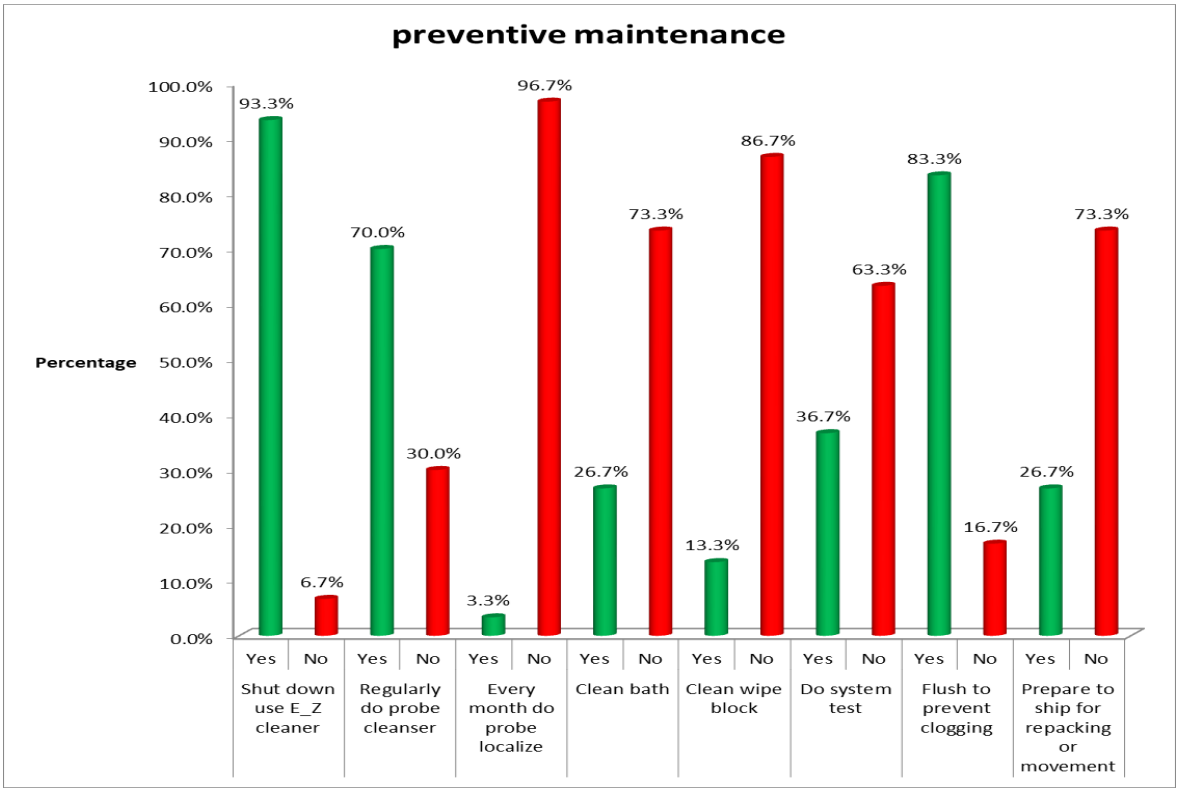


Figure 5.7 The preventive maintenance program for auto hematology analyzer instruments in the surveyed medical laboratory.

Table 5.8 and figure 5.7 explain that medical laboratory most 28(93.3) do shut down instrument using E-Z cleanser daily, 21(70%) do probe clean regularly, 29(96.7%) do not do probe localizer position calibration, 22(73.3%) do not clean bath, 19(63.3%) do not do system test ,25(83.3%) perform flush or zap apertures to prevent clogging and 22(73.3%) follow prepare to guide when decide to repacking or move instrument .

5.1.6.3 **Quality control**

Table 5.9 Overview of quality control on surveyed medical laboratories

Quality control variables		Frequency	Percent (%)
Run QC daily or regularly	Yes	9	30.0
	No	21	70.0
Run QC after maintenance	Yes	10	33.3
	No	20	66.7
Run QC after preventive maintenance	Yes	1	3.3
	No	29	96.7
QC out of range do you do corrective action	Yes	2	6.7
	No	28	93.3

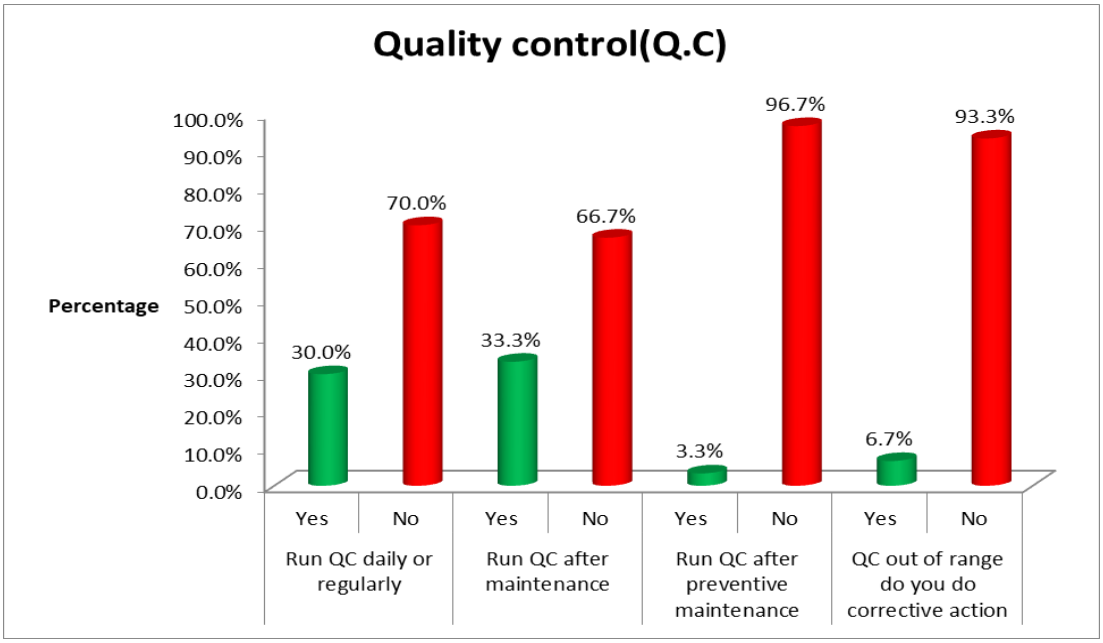


Figure 5.8 Overview of quality control on surveyed medical laboratories

The result from table 5.9 and figure 5.8 illustrate that majority of medical laboratories do not perform preventive maintenance represent 29(96.7%), 28(93.3%)

do not do corrective action if a Q.C out of range, 21(70%) do not proceed Q.C daily or regularly 20(66.7%) do not run Q.C after preventive maintenance.

5.1.6.4 **Calibration**

Table 5.10 Overview of Calibration on surveyed medical laboratories

Calibration variable		Frequency	Percent (%)
Do calibration before first use	Yes	7	23.3%
	No	23	76.7%
Calibrate when change any component	Yes	21	70.0%
	No	9	30.0%
Calibrate in fixed time	Yes	6	20.0%
	No	24	80.0%
Calibrate when QC indicate problem	Yes	9	30.0%
	No	21	70.0%
Do verification after calibration	Yes	1	3.3%
	No	29	96.7%

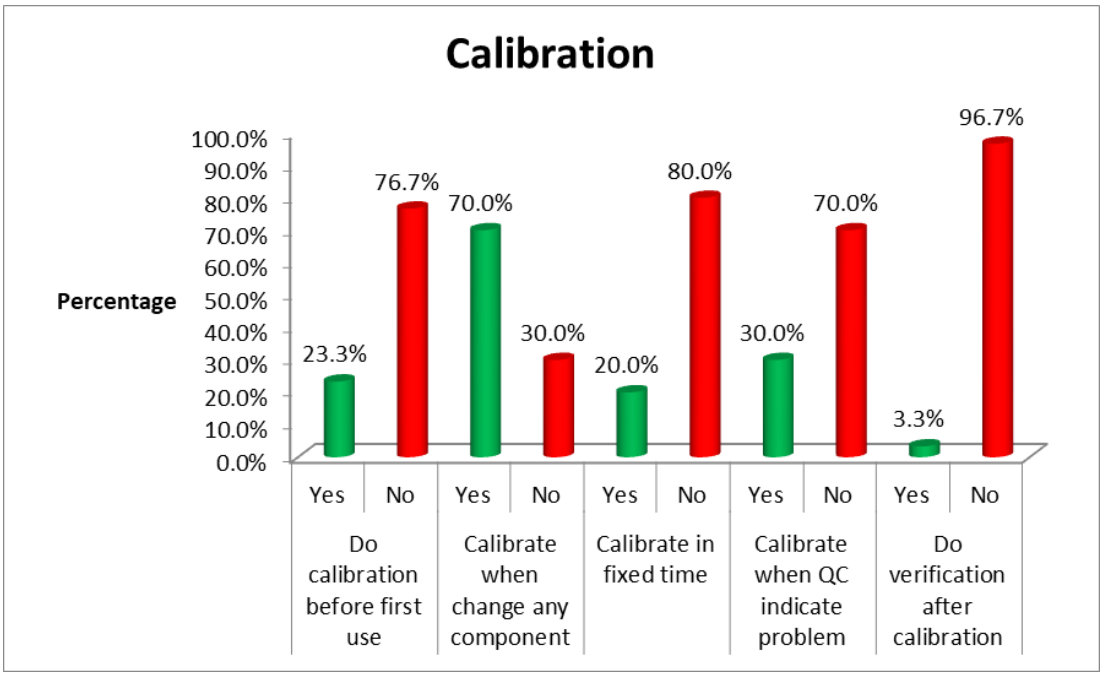


Figure 5.9 Overview of Calibration on surveyed medical laboratories

From table 5.10 and figure 5.9 reflect that 24(80%) of medical laboratory do not perform calibration in fixed interval, 23(76.7) do not due calibration before use instrument for first time and 21(70%) do not calibrate instrument when change any

component. In addition to that 21(70%) do not calibrate instrument if Q.C indicate there is problem and 29(96.7%) due not verify instrument after calibration.

5.1.7 **Environment**

Table 5.11 Illustrates the current environment for auto hematology analyzer when did survey

Environment variables		Frequency	Percent (%)
Ambient temperature error	Yes	23	76.7%
	No	7	23.3%
Appropriate environment	Yes	26	86.7%
	No	4	13.3%
Adequate light	Yes	28	93.3%
	No	2	6.7%
Adequate temperature	Yes	24	80.0%
	No	6	20.0%
Adequate humidity	Yes	30	100.0%
	No	0	0.0%
Adequate space	Yes	17	56.7%
	No	13	43.3%

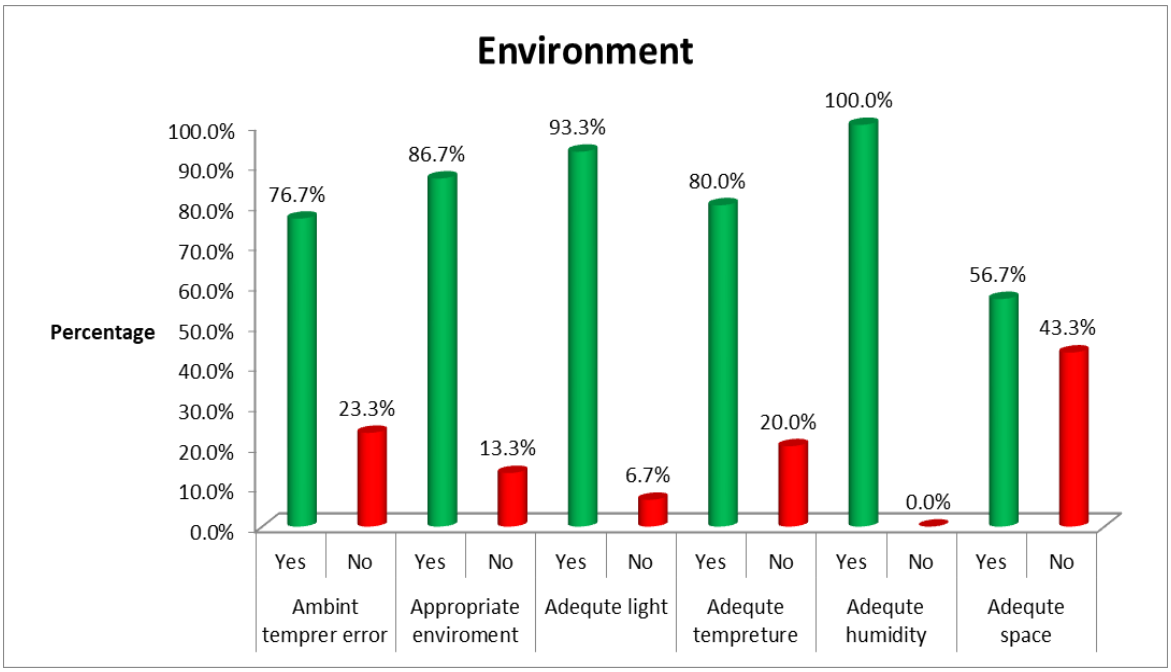


Figure 5.10 Illustrates the current environment for auto hematology analyzer

Table 5.11 and figure 5.10 explain that 30(100%) of auto hematology analyzer instrument use in adequate humidity, 28(93.3) use in adequate light, 24(80%) use

in adequate temperature and 17 (56.7%) use in adequate space .while the total instruments that use in appropriate environment (free from dust, mechanical, vibration, loud noise and electrical interface) were 26(86.7%). it finds that 23(76.7%) of instruments Appeared out on it ambient temperature error.

5.1.8 **Evaluate the performance of auto hematology analyzer (accuracy, precision and reliability)**

An assessment study was conducted to find out the accuracy, precision, and reliability of automated hematology analyzers.

5.1.8.1 **Accuracy**

Table 5.12 Accuracy of auto hematology analyzer measurement for WBC, RBC, HGB, HTC and PLT t on surveyed medical laboratories

Accuracy variable		Frequency	Percent (%)
WBC	(A)_ Accurate	30	100.0%
	(NA)_ Not accurate	0	0.0%
RBC	(A)_ Accurate	24	80.0%
	(NA)_ Not accurate	6	20.0%
HGB	(A)_ Accurate	17	56.7%
	(NA)_ Not accurate	13	43.3%
HTC	(A)_ Accurate	20	66.7%
	(NA)_ Not accurate	10	33.3%
PLT	(A)_ Accurate	17	56.7%
	(NA)_ Not accurate	13	43.3%

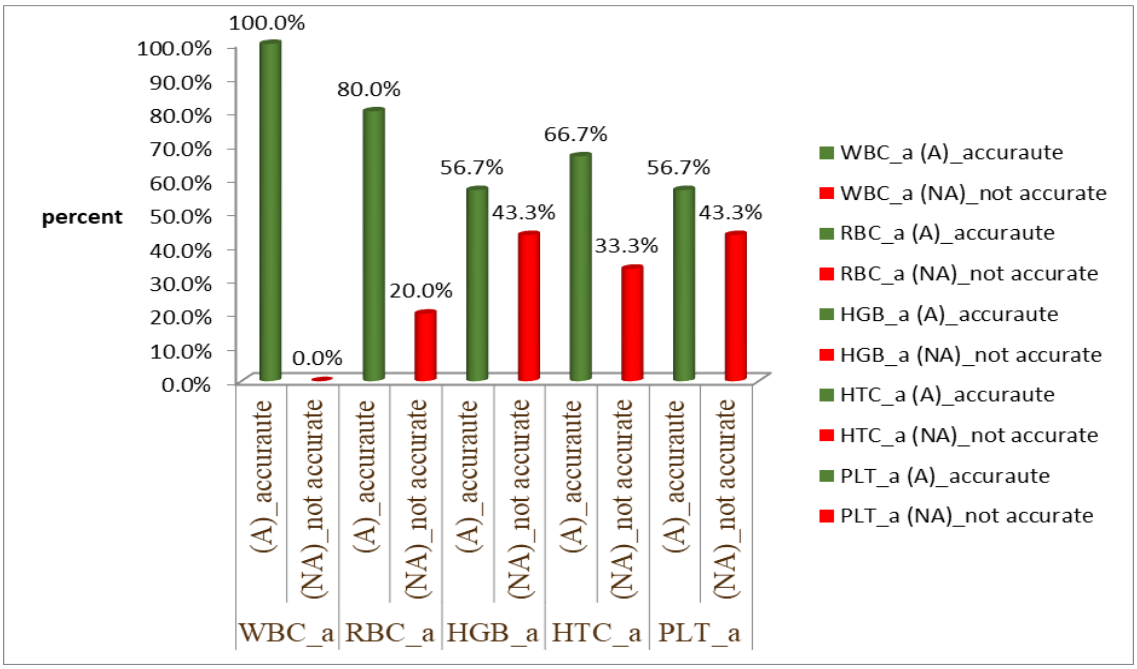


Figure 5.11 Accuracy of auto hematology analyzer measurement for WBC, RBC, HGB, HTC and PLT t on surveyed medical laboratories

Table 5.12 and figure 5.11 explain the accuracy of auto hematology analyzer for WBC, RBC, HGB, HTC and PLT on medical laboratory under the study which it found WBC parameter was accurate on all instrument under study it percent 30(100%), 24(80%) RBC, 20(66.7%) HTC, 17 (56.7%) HGB, and 17(56.7%) PLT.

5.1.8.2 **Precision**

Table 5.13 Precision of auto hematology analyzer measurement for WBC, RBC, HGB, HCT and PLT t on surveyed medical laboratories

Precision variable		Frequency	Percent %
WBC	Precise	24	80.0%
	Not precise	6	20.0%
RBC	Precise	25	83.3%
	Not precise	5	16.7%
HGB	Precise	24	80.0%
	Not precise	6	20.0%
HCT	Precise	25	83.3%
	Not precise	5	16.7%
PLT	Precise	19	63.3%
	Not precise	11	36.7%

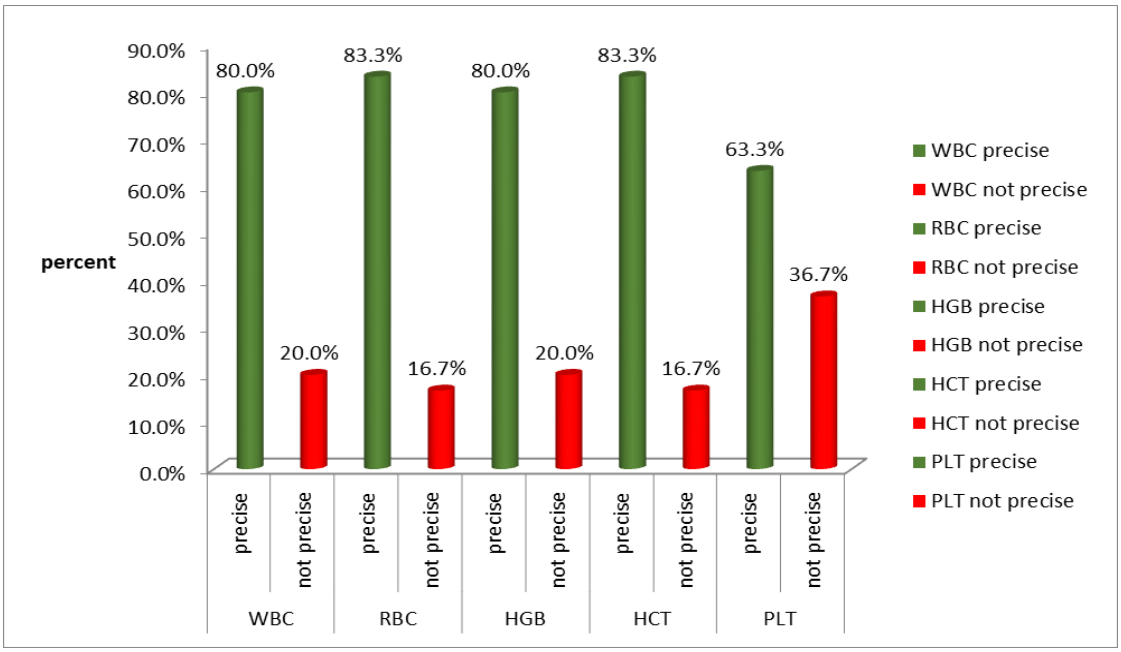


Figure 5.12 Precision of auto hematology analyzer measurement for WBC, RBC, HGB, HCT and PLT on surveyed medical laboratories.

Table 5.13 and figure 5.12 illustrate the precision of auto hematology analyzer for WBC, RBC, HGB, HTC and PLT on medical laboratory under the study it found 25(83.3%) RBC, 25(83.3%) HTC, WBC 24(80%), 24(80%) HGB, and 19(63.3%) PLT.

5.1.8.3 **Reliability**

Table 5.14 Reliability of auto hematology analyzer measurement for WBC, RBC, HGB, HCT and PLT on surveyed medical laboratories

Instrument status	Frequency	Percent (%)
Reliable	4	13.3
Not Reliable	26	86.7

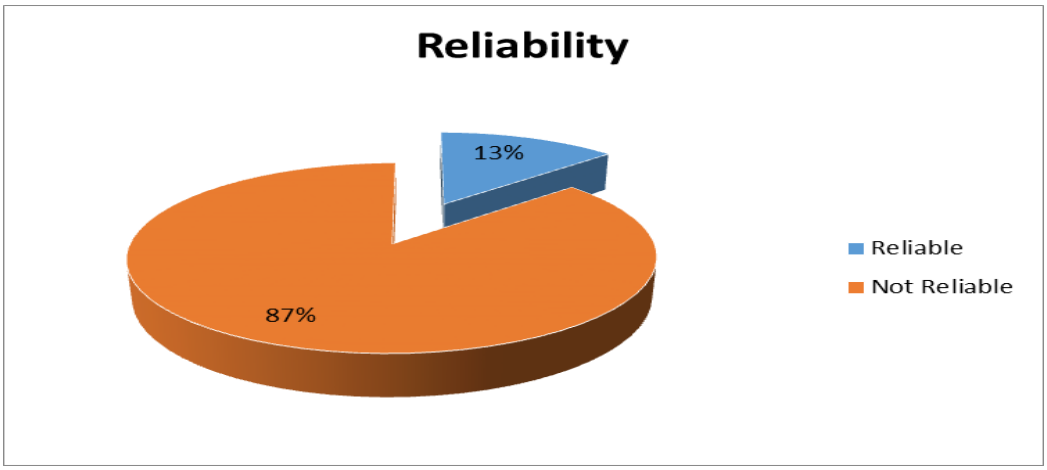


Figure 5.13 Reliability of auto hematology analyzer measurement for WBC, RBC, HGB, HCT and PLT on surveyed medical laboratories

Table 5.14 and figure 5.13 clarify that 4(13%) of auto hematology analyzers were reliable while all other were not reliable.

5.1.9 Relationship between some parameters of auto hemato-logical analyzer results with some factors

5.1.9.1 Relationship between the accuracy, precision and reliability of results of hematological analyzer with machine environment

Table 5.15 The relationship between auto hematology analyzer performances and machine environment.

Auto hematology analyzer performance		Ambient temperature error		Appropriate environment		Adequate temperature		Adequate humidity		Adequate space	
		R	P. value	R	P. value	R	P. value	R	P. value	R	P. value
Reliability		-0.015	0.935	0.154	0.417	0.196	0.299	.a		0.145	0.444
Accuracy	WBC	.a		.a		.a		.a		.a	
	RBC	-0.079	0.679	-0.196	0.299	-0.250	0.183	.a		0.067	0.724
	HGB	0.313	0.092	0.251	0.182	0.235	0.210	.a		-0.086	0.651
	HTC	-0.056	0.770	-0.069	0.716	0.177	0.350	.a		-0.190	0.314
	PLT	-0.164	0.385	-0.145	0.444	0.067	0.724	.a		0.186	0.326
Precision	WBC	0.118	0.534	0.049	0.797	-0.042	0.827	.a		-0.101	0.596
	RBC	-0.035	0.853	0.088	0.645	0.000	1.000	.a		0.331	0.074
	HGB	-0.276	0.140	.539**	0.002	0.583**	0.001	.a		0.404*	0.027
	HTC	-0.035	0.853	0.088	0.645	0.224	0.235	.a		0.331	0.074
	PLT	-0.256	0.172	0.109	0.568	0.484**	0.007	.a		0.451*	0.012

Table 5.15 illustrate the correlation analysis that was done to look relationship between the accuracy, precision and reliability of results of auto hematological analyzer with machine environment of parameters (WBC, RBC, HGB, HTC and PLT).it found that appropriate environment with the standardized coefficient of r= 0.539,(p=0.002 <0.01) was significantly effect on HGB precision , adequate temperature with the standardized coefficient of r=0.484 , (p=0.007 <0.01) with PLT and r=0.583 and (p=0.001<0.01) meaning that the temperature of instrument significant effect on HGB and PLT precision . In addition to an adequate space of r=0.451 and (p=0.012<0.05) was significantly effect on PLT precision and with r=0.404 and (p= 0.027 < 0.05) significantly effect on HGB precision.

5.1.9.2 **Relationship between the accuracy, precision and reliability of results of hematological analyzer with personal**

1. The effect of user training and some other factors on the accuracy, precision and reliability of results of hematological analyzer

Table 5.16 The relationship between auto hematology analyzer performances and users training and other parameters.

Auto hematology analyzer performance		User training		Operation		Standard check		preventive maintenance		QC		Calibrating	
		R	P. value	R	P. value	R	P. value	R	P. value	R	P. value	R	P. value
Reliability		-0.230	0.222	-0.213	0.259	0.000	1.000	0.240	0.202	-0.205	0.278	-0.019	0.921
Accuracy	WBC	.[a]		.[a]		.[a]		.[a]		.[a]		.[a]	
	RBC	0.084	0.660	.[a]		0.298	0.110	0.111	0.559	-0.174	0.358	0.064	0.736
	HGB	-0.191	0.311	.[a]		-0.090	0.635	.456[*]	0.011	0.164	0.387	-0.201	0.287
	HTC	-0.237	0.208	.[a]		0.000	1.000	.393[*]	0.032	0.000	1.000	0.000	1.000
	PLT	-0.191	0.311	-0.016	0.934	0.030	0.875	-0.217	0.250	0.086	0.652	-0.136	0.473
Precision	WBC	-0.056	0.770	-0.024	0.901	-.447[*]	0.013	0.111	0.559	-0.077	0.685	0.145	0.446
	RBC	0.225	0.233	-0.106	0.579	-0.040	0.834	0.248	0.186	.363[*]	0.049	0.215	0.253
	HGB	-0.056	0.770	-0.260	0.166	-0.224	0.235	0.111	0.559	-0.077	0.685	-0.337	0.068
	HTC	0.225	0.233	0.021	0.912	-0.040	0.834	0.149	0.432	-0.052	0.785	0.215	0.253
	PLT	-0.104	0.584	-0.003	0.986	-0.155	0.414	0.131	0.491	0.136	0.472	0.020	0.916

**. Correlation is significant at the 0.01 level (2-tailed).

*.Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant

Table 5.16 shows the correlation analysis between the accuracy, precision and reliability of results of auto hematological analyzer with user training and other factors .it found Standard check was significantly effect on precision of WBC with coefficient r= -0.447(p=0.013 <0.05) and preventive maintenance was significantly effect on accuracy of HGB and HTC with coefficient r = 0.456(p= 0.011<0.05) and r= 0.393(p = 0.032 <0.05) in series .in addition to Q.C significantly effect on precision of RBC with r = -0.363 (p=0.049 <0.05).

2. The effect of user experience on the accuracy, precision and reliability of results of hematological analyzer

Table 5.17 The relationship between auto hematology analyzer performances and user experience.

Auto hematology analyzer performance		User experience	
		R	P. value
Reliability		-0.199	0.292
Accuracy	WBC	.a	
	RBC	-0.137	0.471
	HGB	0.011	0.956
	HTC	-0.221	0.241
	PLT	-0.068	0.720
Precision	WBC	-0.039	0.838
	RBC	0.175	0.356
	HGB	-0.234	0.213
	HTC	-0.140	0.462
	PLT	0.184	0.331

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 5.17 clarifies that there were no correlation between the accuracy, precision and reliability of results of auto hematological analyzer and the user experience. Table shows that (p valve >0.05) for all variables.

5.1.10 Relationship between the performance of auto hematology analyzer and the installation date

Table 5.18 Relationship between the performance of auto hematology analyzer and the installation date (duration of use)

Auto hematology analyzer performance		Installation date	
		R	P. value
Reliability		-0.201	0.286
Accuracy	WBC	A	A
	RBC	-0.085	0.653
	HGB	0.069	0.717
	HTC	0.29	0.12
	PLT	-0.069	0.717
Precision	WBC	0.00	1
	RBC	-0.046	0.81
	HGB	-0.043	0.823
	HTC	0.229	0.223
	PLT	-0.035	0.852

**. Correlation is significant at the 0.01 level (2-tailed).

*.Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant

The correlation analysis was done in order to estimate the relationship between independent variables of auto hematology performance and installation date. These independent variables include accuracy, precision and reliability of parameters (WBC, RBC, HGB, HTC and PLT). Table 5.18 shows that all variables results were statistically insignificant because (P. value> 0.05) meaning that instrument duration of use insignificant affect auto hematology analyzer performance.

5.2 Discussions

This study explored the effect of some parameters on the performance auto hematology analyzer in Khartoum state.

5.2.1 User training

From Table 5.4 as figure 5.3 we founded that 26(86.7%) of user were trained on auto hematology analyzer while 4(13.3%) of it they know the right way to run control or Q.C .That means trained user do not have adequate training on it they know only how to run sample. On the other hand from table 5.5 and figure 5.4 illustrated that the association of user training with the level of health it founded that hospitals was have a high percent in training and strict to use instrument by trained user 13 (43.3%) while the special laboratories have a high percent on trained engineer 7 (23.3).

5.2.2 Instrument operation procedures

From the table 5.6 and fig4ure 5.4 it conclude that most user it use instrument in good way except on three points which are:

- We found that 29(96.7%) of user use general set up in reading sample it does not set according to the age or gender of patient even it change with both age and sex. the study about Complete Blood Count Reference Intervals and Patterns of Changes Across Pediatric, Adult, and Geriatric Ages in Korea shows that RBC parameters increased with age until adulthood and decreased with age in males, but increased before puberty and then decreased with age in females. WBC and platelet counts were the highest in early childhood and decreased with age. Sex differences in each age group were noted: WBC count was higher in males than in females during adulthood, but platelet count was higher in females than in males from puberty onwards ($P < 0.001$). Neutrophil count was the lowest in early childhood and increased with age. Lymphocyte count decreased with age after peaking in early childhood. Eosinophil count was the highest in childhood and higher in males than in females. Monocyte count was higher in males than in females ($P < 0.001$). (Nah**,** Eun Hee et al, 2018).

- 80% of user let reference range as manufacturer setting even though the reference setting of instrument is depend on European or Asian people and they do not have the same characteristic of Sudanese population then the interpretation of result depend on user experience or let the doctor to take decision. The study about complete blood count reference intervals for healthy Han Chinese adults it found that Median and of mean PLT from the Chengdu center were significantly lower than those from other centers. RBC),HGB),and HCT were higher in males than in females at all es .Other CBC parameters showed no significant instrument ,region ,age, or sex dependent difference.(Wu X, Zhao et al, 2015) in addition to other two study in Sudanese population focus on WBC , Hb and RBCs Indices it conclude to Hb level in Khartoum state were lower than the international values also WBCs count in Sudanese are lower than the international.(Taha, Elmutaz, et al, 2018;Awad, Kamal, et al, 2019)

- 70% of user read the sample in other instrument if it gives suspicious result that means it put the main problem of this error in the instrument.

5.2.3 **Instrument and technical knowledge**

5.2.3.1 **Standard check /preventive maintenance**

Majority of laboratories which represent 26 (86.7%) do not have schedule Standard check neither weekly nor monthly but it stick to use manufacturer cleanser when it need to Standard check were represent 28 (93.3%),20 (66.7%) do daily back ground checkup and 16 (53.3) do clean to WBC /RBC transducer in need only after instrument indicate there a problem (error).

As for preventive maintenance the result indicate that most of the laboratories abide by doing shut down using E-Cleanser, doing probe cleanser regular and doing flush aperture to prevent clogging .on other hand they do not do clean bath to avoid contamination, clean wipe block, do system test and prepare to ship for repacking or movement or for long un use of instrument to a void clogging of bath and valve calcification by residual of reagent. This brand does not have a system of programmable preventive maintenance program on it system. Corrective maintenance and preven-

tive maintenance effect on the reliability of medical instrument. (Juliette Fauchet, 2020).

5.2.3.2 **Quality control /calibration**

This study showed that most laboratories do not do quality control or calibration due to absent of doing it.

5.2.4 **Environment**

The survey illustrated that most laboratories have optimal environment except a few of them have a problem in appropriate environment ,adequate temperature adequate space and some instrument give ambient temperature error .But there is no laboratory have a problem in humidity.

5.2.5 **Accuracy, precision and reliability of results of hematological analyzer**

The study showed that the accuracy of the auto hematology analyzer parameters among instruments appear that WBCs were accurate in all instrument while other parameters showed dissimilarity in accuracy in RBCs, HTC, HGB and PLT with percent 80%, 66.7%56.7% and 56.7 respectively.it notice that HGB and PLT were have low present in accuracy among the instrument.

As for precision RBCs and HTC were have a high percent of precision among the auto hematology analyzer instruments with 83.3 followed by WBCs and HGB with 80% and PLT with 63.3%. It found that PLT has low percent in precision in parameters between instruments.

The study illustrated that 4 (13.3%) of auto hematology analyzers were reliable .The percent of reliability is very low it means that 86.7% of instruments were not precise and accurate in all parameters.

5.2.6 **The effect of machine environment on auto hematology analyzer performance**

The findings from this study some of instrument environment factors has significant effect on precision of PLT and HGB. Appropriate environment significantly effect on HGB precision, adequate temperature effect on PLT precision and

adequate space around the instrument was impact on HGB .Appropriate environment, adequate temperature and adequate space surround instrument factors are effect directly in the temperature of instrument which lead to ambient temperature error and the instrument resume measuring the sample without any interrupted in the measuring process.

Previous studies which were focus on blood sample condition and the effect of temperature and time on the stability and reliability of the result of auto hematology analyzer showed that PLT significantly effect by temperature and time with some other parameters.it found that meaningful bias was observed for PLT at 37°C in 3hr, RBC/PLT at 37 °C and 4 °C in 6 hr. in addition to PLT at 24°C and RBC at 37°C in 24hr (Daves, Massimo and et al, 2015).

Other study was done by Gunawardena, Dammika, and et al (2017). related to reliability of parameters of complete blood count with different storage conditions showed that WBC, RBC and HGB were stable at all three temperatures up to 48 hr. while HTC change in 23±2°C and 31±2°C and PLT a significant decline at all study temperatures up to 48 hr. it should be performed within 6 hr. of the post-collection time. From these two studies some parameters can affect by temperature during a period of time PLT, RBC and HTC at 4 °C, 37°C, 23±2°Cand 31±2°C in different period of time.

5.2.7 The effect of personal (user training and other factors) on auto hematology analyzer performance

The result of this study indicate that user training, operation of instrument and the calibration do not effect on the precision and accuracy of auto hematology analyzer parameters while Standard check significantly effect on the WBC precision, preventive maintenance significantly effect on the HGB &HTC accuracy and QC significantly effect on RBC precision which lead to un reliable result followed by risk of wrong diagnostic in addition to wrong medication.

The study done by L Peng, Lbai and et al (2008) to evaluate performance of BC-3200 compare with Beckman it demonstrate that the overall performance of BC-3200 is excellent compare with Beckman- coulter AcT (AcT diff2).other study done by Tavany Elisa Santos Maciel,Samuel Ricardo Comar and Miriam Perlingeiro Bel-

trame to evaluate Sysmex (XE-2100D) according to recommendation document H26-A2 of the CLSI it showed excellent analytical performance, and is useful to provide reliable hematology data.

More over many other studies was done to compare between two hematology analyzer of various manufacturers or between auto hematology analyzer with manual .most studies use correlation to investigate auto hematology analyzer performance and one use interclass correlation. (Saadet Kader,2020; Aliyu A. Babadook ,et al ,2019 ; Hwan Tae Lee,et al,2016; Olga Ciepiela,2046)

Verbrugg, Huisman (2015) was done verification and standardization of blood cell counters for routine clinical laboratory tests it conclude that verification and validation process can be done by the end user by applying intended use criteria .it should include performance analysis of HA on accuracy ,normal range ,back ground ,carry over, lower limit of detection ,clinical report interval and linearity .

5.2.8 The effect of user experience on auto hematology analyzer performance

The results of this study showed user experience did not influences auto hematology analyzer Performance.

5.2.9 The effect of installation date (instrument duration of use) on auto hematology analyzer performance

The results of this study showed instrument duration of use did not influences auto hematology analyzer Performance.

CHAPTER SIX

CNCLUSION AND RECOMMENDATIONS

CONCLUSION &RECOMMENDATIONS

6.1 Conclusion

The result reveals that there is a positive significant relationship between performance of the auto hematology analyzer and the environment elements; such as appropriate environment effect on HGB precision, adequate temperature effect on HGB, PLT precision and adequate space effect on PLT and HGB precision.in addition to some personal parameter significantly effect on it performance such as Standard check effect on WBC precision, preventive maintenance effect on HGB, HCT accuracy ,QC effect on RBC precision. These results indicate that the HGB and PLT parameters are the most affected. The elements such as ambient temperature error, adequate space humidity, user training, instrument operation, and calibration are not significantly effected on it performance. Even though the ambient temperature error has insignificant on the performance of auto hematology analyzer it notes that the PLT was effected in the instruments have this error during measurement. It concluded that the instrument environment has effect on the precision of HGB and PLT while some personal elements effect on precision of WBC, RBC, accuracy of HGB and HCT.

6.2 RECOMMENDATIONS

This study draws baseline information on the effect of some parameters on performance of auto hematology analyzer, since it is a problem effect on the result of instrument and the decision of the doctor and medication which may lead to patient die. Therefore the following recommendations are proposed.

1. Should Do further study using commercial control sample or using reference instrument have specially characteristic.

2. Due to 70% of user repeat the sample in another instrument when doubt about result it recommended that post result assessment.

3. It can do further analysis of data

4. The manufacture should add optional or mandatory system of programmable preventive maintenance program on it system

5. Khartoum state authority and ministry of health is advised to:

   a. Take appropriate steps for providing and activate quality and monitoring units for medical instrument for regularly assessment and grant the health facility accreditation certificates in addition to take an action for Irregularities.

   b. Produce locally quality control sample to use for QC and calibration of instruments which is conducive in terms of ensure the Standardization of measurement in a new and old using instruments.

   c. Since preventive maintenance can enhance the performance of instrument it should obligate health facility to do it increase the reliability of measurement and efficiency of instrument which lead to reducing medical errors related to medical instrument and safe patient.

6.Future research can be done on other manufacturers on same or other state and can be compared with the findings of this study.

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APPENDICES

APPENDICES

Appendix A: Operating environment

Operating temperature: 15 ℃ - 35 ℃.

Optimal operating temperature: 15 ℃ - 30 ℃.„

Relative humidity: 30 % - 85 %.

Appendix B: Space Requirements

At least 28 cm on each side

At least 10 cm behind for cabling and ventilation.

Appendix C: Normal background

Parameter	Background result
WBC	≤ 0.3 × 109 / L
RBC	≤ 0.03× 1012/ L
HG	≤ 1 g / L
HTC	≤ 0.5 %
PLT	≤ 10 × 109 / L

Appendix D: Precision

Parameter	Precision（CV%）
WBC	≤ 2.5
RBC	≤ 2.0
HG	≤ 1.5
HTC	≤ 2.5
PLT	≤ 5.0

Appendix E: Carryover

Parameter	Carry over
WBC	≤ 0.5 %
RBC	≤ 0.5 %
HG	≤ 0.5 %
HTC	≤0.5
PLT	≤ 1 %

Appendix F: Accuracy

Parameter	Accuracy
WBC	±1
RBC	±0.24
HG	±0.6
HTC	±2
PLT	±40

Appendix G: in house survey (part one)

In house survey use to assessment the effect of some parameters on auto hematology analyzer result

Many factors may effect on the result of auto hematology analyzer results here we focus on the effect of the instrument on the result .the practical side of the project have two part first past is questionnaire which fill by user and the second part is study on the device which do by the researcher

Date…… In house survey number: .....

What is the level of Health facility?

1. Hospital ( ) 2. Health center ( ) 3. Dispensary ( ) 4.Specil lab ( )

Part one: fill by user

Section A: personal:

I. staff competency information's:

No	Questions/Statements	Choices	Responses
1.	Sex	1. Male 2. Female	1. Male ( ) 2. Female ( )
2.	Age		( ) Years
3.	Qualification	1. Diploma 2. Bachelor's degree 3. Master's degree 4. Doctoral degree	1. Diploma ( ) 2. Bachelor's degree ( ) 3. Master's degree ( ) 4. Doctoral degree ( )
4.	Years of Experience	Number of years	( ) Years

II. User training:

No	Question	Yes	No
5.	Has everyone (staff and supervisors) received adequate training in the safe use of the equipment?		
6.	Is the use of the equipment restricted to those staff trained to use it?		
7.	Are written instructions for the safe use of the equipment available?		
8.	Has staffs maintaining the equipment been trained?		
9.	Do you know the right way to run control or do Q.C?		

III. Operation:

No	Questions	Yes	No
10.	Is the equipment being used in accordance with the manufacturer's instructions?		
11.	Before turn instrument on should check reagent, connection and etc. Do you inspect the instrument before turn on? (reagent, connection, cord to see are not broken and the power cord properly plugged in)		
12.	When you install new reagent it recommends to do prime .Do you do prime when you install new reagent? (diluent, rinse, lyse)		
13.	When you change any reagent do you run back ground check up to see if it result meet the requirement?		
14.	Any manufacturer recommends using manufacture reagent even if the instrument is open system .Do you use the manufacturer specified reagent? (To provide optimal system performance)		
15.	Do you set the reference range depend on the patient or use general set up? (General reference set up for patient > 12 years)		
16.	Do you change the reference range depend on the characteristic of our local population or study do in Sudanese person?		
17.	When you run sample do you be sure the system status area display ready and count mode display (whole) or (pre diluted) and there is no error message?(it give unreliable result)		
18.	Do you mix sample before aspirate?		
19.	If the sample measurement is greater than the maximum range of the instrument or less than the lowest range of the instrument. What is the procedure followed to measure it?		

Section B: Instrument and Technical knowledge:

1. Instrument information :

No	Questions/Statements	Choices	Responses
20.	Instrument installation date	Date /month/year	…/……/……
21.	instrument Duration use	Years Number of years	( ) Years

2. Standard check :

No	Question	Yes	No
22.	Standard check should schedule as manufacturer guide line. Do you have schedule Standard check :?		

23.	Daily check-up for back ground if it in the permissible range (limit) set by manufacturer is necessary.do you do it daily?		
24.	The sample valve needs to clean weekly. Do you clean it weekly?		
25.	Do you clean WBC, RBC and transducer aperture when needed using manufacture cleaner?		
26.	If you use other cleaner mention it ……..		
27.	Waste chamber need to clean monthly by downing consequence rinse.do you clean waste chamber monthly?		

3. Preventive maintenance:

No	Question	Yes	No
28.	When you want to shut down instrument it should press shut down key and aspirate the cell clean. Do you use E-Z cleanser daily in shutting down? (It is necessary to clean bath automatically then switch off)		
29.	Regularly should do probe clean by using probe cleanser. Do you do? (It does every 3 week if they flow shut down process, and number of sample tests reach 300 samples by whole blood mode or 150 samples by pre diluted mode or every week if does not use specific shut down by using E-Z cleanser)		
30.	Do you do probe localizer position calibration every month?		
31.	Do you clean the bath? (To avoid bath contamination)		
32.	Do you do clean wipe block when needed? By what?		
33.	Do you do system test?		
34.	To unclog the apparatus or prevent clogging we need to do flush apertures (zap apertures) by press [flush] .do you do it?		
35.	When you decide to repacking or move instrument do you follow prepare to ship guide?		

4. Quality control (Q.C):

No	Question	Yes	No
36.	The Q.C should run daily before run sample or regularly. Do you do it daily or regally?		
37.	It necessary to run Q.C after maintenance carried out in the instrument or after broken down. Do you do it?		
38.	Q.C should run after preventive maintenance .Do you do it?		
39.	If the Q.C is out of range. Do you make corrective action? (By checking SD/Mean of Q.C)		

5. Calibration:

No	Questions	Yes	No
40.	When you install analyser in the first time of use it need calibration .Do you calibrate it?		
41.	When certain major component of the analyser changed. Do you calibrate?		
42.	Calibration the instrument should do in fixed in travel at least one in year Do you do it?		
43.	If the Q.C results indicate there may be problem. Do you do calibrate?		
44.	Do you do verification after successful calibration? (Verification do by run Q.C, repeat precision, carry over, accuracy check)		

Section C: Environment:

No	Questions	Yes	No
45.	Does your device give ambient temperature abnormal error?		
46.	Is the equipment only used in an appropriate environment (free from dust ,mechanical vibration ,loud noise and electrical interference)		
47.	Adequate temperature?		
48.	Adequate humidity?		
49.	Adequate seating and space around the machine to allow safe and easy access? (At least 28 cm on each side and 10cm behind for cabling and ventilation)		

THANK YOU FOR YOUR PARTICIPATION

Appendix H: in house survey (part two)

Questionnaire to assessment the effect of some parameters on auto hematology analyzer result

Device model: …… Device serial number: ……

Device manufacture data …… Origin of the device: ……

Part two: fill by researcher

Section A: Checkup the device, room temperature and humidity measurement:

No	Question	Yes	No
1.	Is the histogram start and end in the baseline for RBC, PLT, and WBC?		
2.	Is the system status in the range?		
3.	Is the valve test passed for all valves?		
4.	Is the system test passed?		
5.	Is the hemoglobin (HGB) blank voltage in the range (3.4v to 4.8v)?		

1.	Humidity =					
	Average humidity=					
2.	Temperature =					
	Average temperature =					

Section B: Study:

- **Back ground :**

Description	Measurement	Back ground acceptable range	Result
WBC			
RBC			
HGB			
HCT			
PLT			

❖ **Precision: Precision study performed the analyzes using a blood sample**

Times	WBC	RBC	HGB	HCT	PLT
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
Mean					
SD					
CV%					
Acceptable CV%					
Result					

❖ **Carry over:**

Times	Sample run (firstly run sample 2 times)					Diluent run(blank) (secondly run diluent 3 times)				
	WBC	RBC	HGB	HCT	PLT	WBC	RBC	HGB	HCT	PLT

Calculate carry over using formula: ((blank 1-blank 3)/sample 2)*100%

Description	Calculated carry over	Carry over acceptable range	Result
WBC			
RBC			
HGB			
HCT			
PLT			

- **Accuracy: By using control or sample measure on reference device**

Description	Measurement value	Acceptable measurement	Result
WBC			
RBC			
HGB			
HCT			
PLT			