بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Comparison between Two Different Machines for Estimation of Complete Blood Count in Khartoum Teaching Hospital

مقارنة بين جهازين مختلفين لقياس تعداد الدم الكاملة في مستشفي الخرطوم التعليمي

A dissertation submitted for partial fulfillment for the degree of M.Sc in Medical Laboratory Science (Hematology and Immunohematology)

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قال تعالى:

[وَابْتَغِ فِيمَا آتَاكَ اللَّهُ الدَّارَ الْآخِرَةَ وَلَا تَنسَ نَصِيبَكَ مِنَ الدُّنْيَا وَأَحْسِن كَمَا أَحْسَنَ اللَّهُ إِلَيْكَ وَلَا تَبْغِ الْفَسَادَ فِي الْأَرْضِ إِنَّ اللَّهَ لَا يُحِبُّ الْمُفْسِدِينَ]

صدق الله العظيم

سورة القصص: الآية (77)

Dedication

To my father, my mother, my husband, my brothers and my teachers whom love and support me made me able to continue my study in the field of medical laboratory and face all challenges that came on my way……..

Acknowledgment

First of all my humble thanks to almighty Allah without his blessing and benevolence it would not have been possible for me to achieve this goal, special praises to his holly prophet Mohammed who is forever a ray of knowledge and guidance for whole humanity.

I wish to express my deepest gratitude to my supervisor **Prof. Babiker Ahmmed Mohammed** whose continuous advices and encouragement was a driving force for me to accomplish this work.

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Abstract

This is a descriptive analytical study, conducted in Khartoum   Teaching Hospital in Khartoum state from research laboratory and emergency laboratory during the period from May 2015 to March 2016.

The study was performed to estimate CBC (complete blood count) by using two sysmexes KX-21n , in aim to compare between this machines, one hundred blood samples were collected in EDTA container (2.5 ml), CBC estimation was carried by sysmex KX-21n (semi automated method), and the results were analyzed using SPSS computer programme.

The results indicated that: Hb (12.7 ± 1.9g/dl), Hct (38.5 ± 5.7%), RBCs (4.64 ± 0.72×10^{12} /l), MCV(82.9 ± 8.7 fl), MCH(30.1 ± 2.7pg), RDW-SD (44.8 ± 5.5), RDW-CV (14.7 ± 2.3), TWBCs (6.3 ± 2.2×10^9 /l), lymphocyte count (35.5 ± 12.2%), neutrophil count (53.1 ± 12.9%), MXD count (eosinophil, monocyte, and basophil) (10.9 ± 4.3%), absolute lymphocyte count (2.1 ± 0.77 ×10^9 /l), absolute neutrophil count (3.5 ± 1.9×10^9 /l), absolute MXD count (eosinophil, monocyte, and basophil) (0.6 ± 0.25×10^9 /l), Plt (256 ± 102×10^9 /l), PDW (12.3 ± 1.8), MPV (9.9 ± 0.9), and P-LCR (24.5 ± 6.9) in the sysmex of the research laboratory while the results in the sysmex of the emergency laboratory showed that: Hb (12.6 ± 1.9g/dl), Hct (39.7 ± 7.1 %), RBCs (4.63 ± 0.72×10^{12} /l), MCV (84.8 ± 9.2fl), MCH (27.4 ± 2.8pg), RDW-SD (45.6 ± 6.6), RDW-CV (14 .6 ± 2.3), TWBCs (6.2 ± 2.3×10^9 /l), lymphocyte count (36.5 ± 13.2%), neutrophil count (53.3 ± 13.5%), MXD count (eosinophil, monocyte, and basophil) (10.0 ± 3.6%), absolute lymphocyte count (2.1 ± 0.83×10^9 /l), absolute neutrophil count (3.5 ± 2.0×10^9 /l), absolute MXD count (eosinophil, monocyte, and basophil) (0.6 ± 0.25×10^9 /l), Plt (264 ± 104×10^9 /l), PDW (12.2 ± 1.8), MPV (9.8 ± 1.3), and P-LCR (25.5 ± 9.4), and this results explained that there were   insignificant differences between reading in both two sysmexes (P.value 0.6, 0.2, 0.9, 0.1, 0.2, 0.4, 0.8, 0.8, 0.5, 0.9, 0.5, 0.8, 0.9, 0.1, 0.6, 0.8, 0.6, and 0.4) respectively, except in the results of

MCHC (33.0 ± 2.7%) in the sysmex of the research labotatory and MCMC (32.0 ± 2.2%) in the sysmex of the emergency laboratory there were significant differences (P.value 0.004).

المستخلص

هذه دراسة وصفية تحليلية ، اجريت في مستشفى الخرطوم التعليمي في ولاية الخرطوم من معمل البحث ومعمل الحوادث في الفترة من مايو 2015 حتي مارس 2016، وهدفت هذه الدراسة لتقدير النتائج لصورة الدم الكاملة باستخدام جهازين مختلفين من نفس النوع (sysmex KX21n)، بهدف المقارنة بين هذين الجهازين.

تم اخذ عدد مائة عينه في وعاء يحتوي علي مادة مانعة لتجلط الدم وكان مقدارها 2.5 مل ، لقد تم اجراء عملية فحص صور الدم الكاملة باستخدام الجهاز الشبه الي sysmex KX21n، وقد تم تحليل النتائج بواسطة الحاسب الالي باستخدام برنامج الحزم الاحصائية للمجتمع SPSS.

واظهرت النتائج ان: ليس هنالك اختلافات ذات دلالة معنوية عند مقارنة النتائج بين جهاز السيسمكس في معمل البحث حيث وجد أن الهيموقلوبين (12.7 ± 1.9g/dl)، الدم المكدس (38.5 ± 5.7%)، عدد كريات الدم الحمراء (4.64 ± 0.72×10^{12} /l)، متوسط حجم الخلية الحمراء (82.9 ± 8.7 fl)، متوسط تركيز خضاب الدم للخلية الحمراء (30.1 ± 2.7pg) ، معدل انتشار الخلية الحمراء (44.8 ± 5.5)، عدد كريات الدم البيضاء (6.3 ± 2.2×10^9 /l)، النسب المئوية للخلايا الليمفاوية (35.5 ± 12.2%)، النسب المئوية للخلايا العدلة (53.1 ± 12.9%)، العدد المطلق للخلايا الليمفاوية (2.1 ± 0.77 ×10^9 /l) ، العدد المطلق للخلايا العدلة (3.5 ± 1.9×10^9 /l)، عدد صفائح الدم (256 ± 102×10^9 /l)، معدل انتشار الصفائح الدموية (12.3 ± 1.8) ومتوسط حجم صفيحة الدم الواحدة (9.9 ± 0.9) ، و بين نتائج السيسمكس في معمل الحوادث: الهيموقلوبين (12.6 ± 1.9g/dl)، الدم المكدس (39.7 ± 7.1 %)، عدد كريات الدم الحمراء (4.63 ± 0.72×10^{12} /l)، متوسط حجم الخلية الحمراء (84.8 ± 9.2fl)، متوسط تركيز خضاب الدم للخلية الحمراء (27.4 ± 2.8pg)، معدل انتشار الخلية الحمراء(45.6 ± 6.6)، عدد كريات الدم البيضاء (6.2 ± 2.3×10^9 /l)، النسب المئوية للخلايا الليمفاوية (36.5 ± 13.2%)، النسب المئوية للخلايا العدلة (53.3 ± 13.5%)، العدد المطلق للخلايا الليمفاوية (2.1 ± 0.83×10^9 /l)، العدد المطلق للخلايا العدلة (2.0 ± 3.5×10^9 /l) ، عدد صفائح الدم (264 ± 104×10^9 /l) ، معدل انتشار الصفائح الدموية (12.2 ± 1.8)، ومتوسط حجم صفيحة الدم الواحدة (9.8 ± 1.3).

وان هنالك فروقات ذات دلالة معنوية في تركيز خضاب الدم في 100 مل من الدم (2.7% ± 33.0) في معمل البحث و (2.2% ± 32.0) في معمل الحوادث.

Abbreviations

AHAs   Automated Hematology Analyzers

CBC   Complete Blood Count

CD   Cluster of Differentiation

CME   Continuing Medical Education

CPD   Continuing Professional Development

CV   Coefficient of Variation

DLC   Differential Leukocyte Count

EDTA   Ethylene-diamine-tetra acetic acid

EQA   External Quality Control

FNs   False Negatives

FPs   False Positives

Hb   Hemoglobin

Hct   Hematocrit

ICSH   International Council for Standardization in Hematology

IQC   Internal Quality Control

LIMS   Laboratory Information Management System

L-J   Levy –Jennings chart

MCH   Mean cell hemoglobin

MCHC   Mean cell hemoglobin concentration

MCV   Mean cell volume

MPV   Mean platelet volume

PCV   Packed cell volume

PDW   Platelet Distribution Width

QC	Quality Control
RBC	Red blood cell
RDW-CV	Red cell distribution width by Coefficient of variation
RDW-SD	Red cell distribution width by standard deviation
SD	Standard Deviation
SOP	Standard Operating Procedure
TNs	True Negatives
TPs	True Positives
WBC	White blood cell
WHO	World Health Organization

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Chapter one
Introduction and Literature Review

1.1 Introduction:

Management of patients with hematological disorders has become increasingly arduous, requiring the hematologist to acquire unique clinical skills; conversely, major developments in laboratory practice require a high level of technical expertise, especially in handling automated instruments and information technology (Hoffbrand et al, 1999).

The sysmex KX-21n is ideal for clinic satellite laboratory or research testing. The sysmex KX-21n hematology analyzer provides17 reportable parameters including a 3-part WBC differential, plus histograms for RBC, and Plts, it provides a high level of accuracy through the use of automatic floating discriminators.

A complete blood count (CBC), is a blood panel that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals (David and Dugdale, 2012).

This evaluation consists of the components that offers the clinician a variety of hematological data to interpret and review that directly relate to the health of the bone marrow, represented by the numbers and types of cells in the peripheral, the components of the CBC are WBC count, RBC count, HB, PCV, MCV, MCH, MCHC, PLT count, and RDW, DLC (neutrophil ,lymphocyte, monocyte, eosinophil, basoplil), PMV, PDW depending on the type of automated instrumentation used (Ciesla ,2007).

1.2 Literature Review:
1.2.1 The sysmex :

The sysmex KX-21n is performs rapid and accurate analysis of 17 parameters, utilizes same direct current detection method, so it produces accurate results comparable to other sysmex hematology analyzers, also it is compact and fully integrated, ideal as back-up for sysmex 5-part differential systems, and it Fits easily on a laboratory bench or table, sysmex KX21n has quality assurance program and sensitive flagging to support diagnosis by the physician, so it is accurate and reliable, also it is easy in operation and maintenance because it has a simple menus and push-button technology, so minimal training required, and the most important criteria of this machine it is safety and secure because it use non-toxic, biodegradable reagents.

1.2.1.1 Principle of sysmex KX-21 hematological analyzer:

 Blood is highly diluted in a buffered electrolyte solution. The flow rate of this diluted sample is controlled by a mercury siphon or by displacement of a tightly fitting piston. This results in a measured volume of the sample passing through an aperture tube of specific dimensions. By means of a constant source of electricity, a direct current is maintained between two electrodes, one in the sample beaker or the chamber surrounding the aperture tube and another inside the aperture tube. As a blood cell is carried through the aperture, it displaces some of the conducting fluid and increases the electrical resistance. This produces a pulses corresponding to change in potential between the electrodes, and then this pulses can be displayed on an oscillograph screen (Dacie and Lewis, 2011).

1.2.1.2 The related testes are:
1.2.1.2.1 Hemoglobin estimation:

 Hemoglobin is oxygen carry protein within the erythrocytes of the blood it's made of heme and globin, the red pigment of heme provides the red cell color of blood (Stevens, 1997).

Hemoglobin is a conjugated protein of molecular weight 6400 Dalton, consisting of two pairs of polypeptide chain to each of which a heme is attached (Firkin et al, 1989).

Blood normally contains 12-18g/dl of hemoglobin (Cheesbrough, 2006).

1.2.1.2.2 Red blood cell count:

It is screening test for anemia, polythycemia and calculate absolute value normal range (4.5 -5.6$\times 10^{12}$/l) (Dacie and Lewis, 2011).

1.2.1.2.3 Packed cell volume:

Packed cell volume (PCV) can be used as a simple screening test for anemia, as a reference method for calibrating automated blood count systems, and as a rough guide to the accuracy of hemoglobin measurements (Dacie and Lewis , 2011).

1.2.1.2.4 Total white blood cell count:

It is use to detect numerical abnormality of WBCs in peripheral blood normal rang is (4-11$\times 10^{9}$/l) (Dacie and Lewis, 2011).

1.2.1.2.5 Differential leukocyte count:

It is usually performed by visual examination of blood film, expressed as percentage of each type of cell (Dacie and Lewis, 2011).

1.2.1.2.6 Platelet count:

It is use for detect numerical abnormality of platelets in peripheral blood, normal range is (150-450$\times 10^{9}$/l) (Dacie and Lewis, 2011).

1.2.1.2.7 Red blood cell indices:

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC). The MCV is important in evaluation of erythrocyte disorders, the MCH and MCHC are generally not of great value, and the red cell distribution width (RDW) is mathematical description of the variation in RBC sizes, a high RDW indicates greater variation in RBC sizes. They are considerable clinical importance and are widely used in classification of anemia (Dacie and Lewis, 2011).

1.2.1.3 Calibration of sysmex:

Calibration is performed to compensate for any inaccuracies of the pneumatic, hydraulic, and electric systems which will affect analysis results. This is very important in maintaining the system accuracy. Calibration is carried out by entering calibration values into the unit. The sysmex service representative would perform initial calibration of the unit at installation, after installation the operator should make periodical calibration and proper quality control to maintain the accuracy.

Calibration need not be performed in specified intervals, but when QC data varies with time Hb and Hct should be calibrated. When abnormal QC data occur as a result of instrument problem, reagent degradation, or deterioration of control blood, however, did not perform calibration.

For calibration, used five or more samples of fresh normal blood that met the following conditions: blood should be taken from a healthy person who is not taking any medicine and added with appropriate anticoagulant, per-sample whole blood volume to exceed 2 ml, Hb value to exceed 10.0 g/dl, and Hct value to be within 35.5% - 55.5%.

1.2.1.4 Control materials of sysmex:

Quality control checks are performed to monitor an instrument's performance over time. EIGHTCHECK-3WP is the quality control material recommended by sysmex to monitor the performance of the KX-21 analyzer. This product is supplied with three control levels, at least two levels should be run every 8 hours of operation or in accordance to regulations applicable to your laboratory. Quality control material should be run after component replacement or after a service call. The KX-21 has two quality control methods. X Control in which control blood is analyzed twice and the mean of the two is used to evaluate analyzer performance,

and Levy-Jennings Control (L-J). The L-J control uses the data from a single control blood analysis to evaluated analyzer performance (Operators, 1999).

1.2.1.5 Quality specification in hematology: the automated blood cell count:

Quality specifications for automated blood cell counts include topics that go beyond the traditional analytic stage (imprecision, inaccuracy, and quality control) and extend to pre- and post-analytic phases, in these review pre-analytic aspects concerning the choice of anticoagulants, maximum conservation times and differences between storage at room temperature or at 4 degrees C are considered. For the analytic phase, goals for imprecision and bias obtained with various approaches (ratio to biologic variation, state of the art, and specific clinical situations) are evaluated, as result K2EDTA is considered the anticoagulant of choice for automated cell counts. Regarding storage, specimens should be analyzed as soon as possible. Storage at 4 degrees C may stabilize specimens from 24 to 72 h when complete blood count (CBC) and differential leukocyte count (DLC) is performed. For precision, analytical goals based on the state of the art are acceptable while for bias this is satisfactory only for some parameters, in conclusion in hematology quality specifications for pre- and analytical phases are important, but the review criteria and the quality of the report play a central role in assuring a definite clinical value (Buttarello, 2004).

1.2.2 Physiology of blood:

1.2.2.1 Definition of blood:

Blood is specialized connective tissue, which circulate in closed system of blood vessels. The average person has approximately 70 ml of blood per kilogram body weight (70 ml/kg) (Kern, 2002).

1.2.2.2 Blood component:

Approximately 50 to 60 % of the blood volume is liquid, and the remainder is cells. The liquid component called plasma is nearly 90 % water and the remaining 10% protein and other components, the serum is solid part that is separated from

the whole blood without addition of anticoagulant it is same as plasma except that the clotting factors and fibrinogen have been consumed. The cell of blood can be divided into, erythrocyte (red blood corpuscles), leukocyte (white blood corpuscles) of various types, and platelet (Kern, 2002).

1.2.2.3 Function of blood:

The exchange of respiratory gases, carrying oxygen from lung to the tissues and up taking of carbon dioxide CO2 from the tissues locate it to lungs to be exhaled and execrated, blood also transports metabolic wastes to the lung, kidney, skin, and intestine for removed. It also responsible for maintaining acid base balance, and regulation of body temperature and distribute nutritis to the body (Zakarya, 1995).

1.2.3 Complete blood count (CBC):

Complete blood count is very common test uses to evaluate the three major type of cell in blood red blood cells, white blood cells, and platelets (Dacie and Lewis, 2011).

1.2.3.1 Important of CBC:

It is screening test to check some blood disorders "anemia, polycythemia infection and inflammation, it is used to determine the general health status of people as monitoring and follow up the treatment and drugs effects (Dacie and Lewis, 2011). This is one of the most common tests ordered by physicians and can yield valuable information about hematologic and no hematologic illnesses. There are two parts to interpreting this test, interpretation of reported values and review of the blood smear. The reported values are either directly measured or calculated by a machine called coulter counter. The measured values are, RBC number, Hematocrit (HCT) and Hemoglobin (Hb), while the calculated values are, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).In general, MCV is the most useful index and divide the anemias into microcytic, normocytic and macrocytic types. The MCH and MCHC add very little to the information provided by MCV.

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Another piece of useful information on the CBC results is red cell distribution width (RDW). The RDW is an expression of the size distribution spread of the erythrocyte population, it is computed from the RBC histogram and is the co-efficient of variation, expressed in percent of the red cell size distribution. RDW goes up in 95% of cases with iron deficiency and about two thirds of the cases with megaloblastic anemia. Significantly it does not change in anemia of chronic disorder and thalassemias.

Two other terms may be reported in the CBC results, anisocytosis which means there is marked variation in size of red cells, and poikilocytosis means there is a significant variation in shape of red cells.The white cells and platelets are counted by the machine. The white cell differential is also done by the machine but is routinely reviewed by a technologist. The newer machines also give platelet volume (equivalent of MCV for red cells) in the CBC report. Larger platelet volume indicates younger and more active platelets.

1.2.4 Quality control on hematology:

This is the aspects of laboratory function which is especially important to ensure the reliable results, which include, quality assurance which defined as various procedures that are directly concerned with test reliability, clinical reliability which concerned with the using of appropriate test methods or analytical systems, test interpretation, relevant reference ranges, specimen collection and sampling, and provide to the staff health and safety control, professional education and training opportunities, clinical and technical audit, involvement in management with delegated responsibilities, and confidence in the management, and organization and management which include strategic planning, budget control, computer applications, record-keeping, application of up-to-date methods and adherence to mandatory regulations (Hoffbrand et al,1999).

1.2.4.1 Quality control:

It is process for assessing the accuracy and reliability of the test system. In clinical laboratory this is usually accomplished by running specimen with known values or outcomes along with patient unknown to determine if the analytical system is functioning with prescribed boun dories, the basis for end clinical laboratory quality control in program is a written QC policy. This policy should include a description the control materials to be used with each assay frequency of use required documentation as well as criteria for the acceptability of patient results. The quality control policy must also state that when results are unacceptable any patients results that run with those with QC specimen cannot be reported, it should identify a set remedial action that should be instituted.

(Feld, 2001).

Focus on clinical laboratory mistakes and on concepts to design quality into analytical system to reduce or eliminate mistakes and must anticipate the mistakes that are most likely to occur, and design mistake-proofing system (Hinchley, 1997).

Introduction of the computer into laboratory has mode great contribution to the progress of the quality control with quality control materials, such as west grad multiple charts have been widely accepted, but the computer limitation in detects random errors made use to consider a new method of delta check (Jonk et al, 1990).

Laboratory medicine specially clinical chemistry and hematology forerunner in the field of quality control in medicine, nearly all clinical chemistry and hematology laboratories participate in one or more, national or international quality control programs , organized by either professional organization or industry . Laboratories also spend much time and many on internal quality control programs (Leijsae, 1983).

1.2.4.2 Quality assurance:

For reliable test results it is important to ensure precision (i.e. reproducibility within agreed limits when an analysis is performed repeatedly on the same specimen), accuracy (i.e. truth), standardization and harmonization (i.e. comparability of results between laboratories). Different procedures are used in respect of quantitative, semi-quantitative and qualitative tests. Precision in quantitative tests, expressed as coefficient of variation of the measurements (CV %), is relatively easy to achieve. Accuracy is more problematic for most hematology tests, as there are few internationally established reference standards, and standardization is usually based on calibration by arbitrary standard preparations or by using standardized methods. But the most important criterion is harmonization to ensure comparability of measurements between laboratories, for the benefit of both the individual patient, who may be seen at different times in different clinics, and for collaborative studies, such as therapeutic trials, which may be undertaken at different centres. There are five components in a quality assurance program, standardization and instrument calibration, internal quality control (IQC), statistical control with patient data , correlation assessment , and external quality assessment (EQA) (Hoffbrand et al ,1999).

1.2.4.2.1 Standardization:

Most modern analyzers are arbitrary comparators rather than absolute measurement devices. To perform reliably they must be calibrated, thus correct measurements on test samples depend on the accuracy of the value that has been assigned to the calibrator. Generally, calibrators are provided by the instrument manufacturers or are available from commercial companies, their reliability depends on the efforts made by the manufacturers to establish their true value. Control preparations also have assigned values, but these are only approximations, and controls should not be used as calibrators. Wherever possible calibrators should be directly traceable to an international (primary) standard with exactly defined physical or chemical measurement. Where no reference standards are

available, harmonization depends on adherence to written standards and practice guidelines such as those published by WHO and the International Council for Standardization in Hematology (ICSH) (Hoffbrand et al, 1999).

1.2.4.2.2 Internal quality control:

Internal quality control (IQC) is intended to ensure that measurements are sufficiently precise day by day or batch by batch within established limits. There are several methods of IQC which complement each other, the best known method is to test a control sample at intervals alongside the routine specimens, and to plot the results on a Levy–Jennings control chart. This is a linear graph showing the mean and limits of standard deviation (SD) at 1SD and 2SD, and the other method is delta check method and this is refers to comparison between consecutive tests on a patient within 1–2 weeks (Hoffbrand et al, 1999).

1.2.4.2.3 Statistical control with patient data:

This method of control is based on the principle that the overall daily means of the blood count parameters will remain constant, provided that the proportion of different categories of patients in a hospital remains more or less the same. The calculations are adjusted by means of an algorithm to obtain a weighted moving average. This procedure has been incorporated in multichannel analyzers to provide an automated continuously updated control of accuracy. It will detect analytic drift and change in calibration, instrument imprecision will be detected by an increase in the SD (Hoffbrand et al, 1999).

1.2.4.2.4 Correlation assessment:

A control chart will not detect blunders in individual routine specimen, which can only be detected by finding that the test results do not correlate with other laboratory tests or the clinical condition. These discrepancies can be checked by cumulative reports, blood film examination, patient's clinical state, and whether the blood count is consistent with the results of other investigations (Hoffbrand et al, 1999).

1.2.4.3 External quality assessment (EQA):

Although IQC provides continuous vigilance, EQA checks test performance only on random occasions. However, by comparing results from different laboratories it is possible to establish between laboratory and between method performances. Thus EQA schemes have an important educational role, not only for participating laboratories whose performance is under scrutiny, but also for assessing the state of the art overall, for identifying and recommending the best methods for various tests, and persuading participants to abandon unreliable methods. It is a valuable interface between users and manufacturers of instruments, reagents and kits, because the EQA data may indicate an otherwise unrecognized product failure, as well as the problem of lack of harmonization of results when different technologies are used in analysis (Hoffbrand et al, 1999).

1.2.4.4 Pre- and post-analytic control:

Both IQC and EQA are concerned with the actual analytical tests at the laboratory bench. Clinical reliability is concerned primarily with the test results, whether they are useful and can be relied upon for patient care or for health screening. Thus functions that may indirectly influence test reliability must also be taken into account. These include all stages from specimen collection, transport to the laboratory, registration and selection of the most appropriate methods for the required test(s), as well as validation of the results, their interpretation and transmission of a meaningful (and legible) report to the appropriate unit or individual (Hoffbrand et al, 1999).

1.2.4.4.1 Specimen collection:

All hematology tests start with blood collection. It is essential to ensure that specimens are collected by a standardized procedure and reach the laboratory in good condition (Hoffbrand et al, 1999).

1.2.4.4.2 Phlebotomy:

The phlebotomist is responsible for ensuring that all specimens and associated request forms are adequately identified, with appropriate indication of especially hazardous specimens, and these specimens must then be transported to the laboratory without delay, and maintained during transit at an appropriate ambient temperature to minimize deterioration. When a pneumatic delivery system is in use, it must be checked on installation to ensure that it has no untoward effect on the specimens and that there is no leakage. As blood cell morphology requires films to be made and fixed as soon as possible, if specimens are sent from a distance it may be desirable to have the films prepared and fixed at the time of blood collection (Hoffbrand et al, 1999).

To take account of these various factors the International Society of Hematology and International Council for Standardization in Hematology have jointly published a standard for Specimen collection, storage and transmission to the laboratory for hematological tests (Hoffbrand et al, 1999).

1.2.4.4.3 Pre-analytic proficiency:

The first clerical act is to record the time of arrival of specimen in laboratory. The container should then be checked for leakage, insufficient volume or incorrect anticoagulant for the requested test(s). The request and specimen are given a laboratory reference number and marked either for immediate attention ('stat'),or as 'urgent' or 'routine' and sent to the appropriate section(s) of the laboratory for testing (Hoffbrand et al, 1999).

1.2.4.4.4 Post-analytic proficiency:

After the tests have been carried out, the following procedures are required to ensure proficiency in the post-analytic phase, processing of results for transcription on to report forms, immediate scrutiny of urgent results with issue of provisional report and its delivery to the requesting clinician, assessment of the significance of results in the context of established reference values and decision for further tests, transmission of final report without unreasonable delay to the location indicated on

the request form, and contact with users to ensure that the reports arrive in due time for optimal use during clinical management and that the results are presented in a clear and unambiguous form (Hoffbrand et al, 1999).

1.2.4.4.5 Room temperature:

It is generally define as the ambient air temperature whatever environment being used for a given procedure. More specifically it is define as 20-25C (68-77F) as some ambient temperature , by nature do not fall within this range , and protocols calling for steps to be performed at RT require that temperature do not fall below 18C (64F) , and don not exceed 27C (80F) (Ganio, 2008)

1.3 Rationale:

There are differences in CBC results among different laboratories, and this may be due to the uses of different type of automated instrument for estimation, the environment of the laboratory, and the technical staff which may not evident with generation of Q.C system of the sysmex.

1.4 Objective:

1.4.1 General objective:

To comparison between two sysmexes in Khartoum Teaching Hospital.

1.4.2 Specific objective:

-To determine if there is any difference in complete blood count results when measured by using two sysmexes KX-21n in the Khartoum Teaching Hospital from research laboratory and emergency laboratory.

Chapter Two
Materials and Methods

Chapter 2

Materials and methods:

2.1 Study design:

It is descriptive analytical study, conducted in period from May to June 2015 to March 2016,to estimate CBC in aim to compare between two sysmexes KX-21n.

2.2 Study area:

This study was conducted in Khartoum Teaching Hospital in Khartoum state from research laboratory and emergency laboratory.

2.3 Study population:

Samples were obtained from patient who requested for routine complete blood count (CBC) investigation in Khartoum Teaching Hospital.

2.4 Sample size:

The sample size was set as one hundred samples which selected randomly, (2.5) ml of blood in EDTA anticoagulant.

2.5 Ethical consideration:

The consent was taken from the general manager of laboratories in Khartoum Teaching Hospital after being informed with all detailed objectives of the study.

2.6 Data analysis

Data were analyzed by SPSS computer program using independent t test. The significant P value was set at $P \leq 0.05$.

2.7 Materials and equipments:

Plastic EDTA containers, sterile cotton, Alcohol (70%), Disposable syringes, Tourniquet, Sysmex KX-21, Diluents cell pack, WBC and HB reagents (Stromatolyzer), and Detergent cell clean.

2.8 Methods:

2.8.1 Collection procedures:

Person was on comfortable sitting, a tourniquet applied above elbow, and superficial ante-cubical form vein was identified. The skin was sterile with 70% alcohol and allowed to dry. Syringe needle inserted correctly into the vein, and 2.5ml of blood was taken from the ante-cubital vein of the forearm, tourniquet was released needle removed, and 2.5ml of blood was drained into EDTA container and mixed with anticoagulant gently for several times.

ʿDacie and Lewis, 1995).

2.8.2 CBC measurement:

1-The instruments were checked up for the sufficient of the solution also checked electric power supply, machine has full battery and earthed connected then power key was pressed on.

2- Samples were well mixed and entered to probe then the start switch was pressed, when LCD screen was displayed analyzing the sample removed, 30 sec and then the result were printed out.

2.8.2.1 CBC components:

-**Hemoglobin:**

Analysis principle non – cyanide hemoglobin analysis method volume (gram) of hemoglobin \1dl of whole blood.

-**HCT (hematocrit value):**

Analysis principle RBC pulse height detection method

Ratio (%) of whole RBC volume in whole blood.

-**RBC (red blood cell):**

Analysis principle DC detection method.

-**MCV (mean cell volume):**

Mean cell volume (fl) in whole blood.

MCH (Mean cell hemoglobin):

Mean cell hemoglobin (Pg) per RBC.

-**MCHC (mean cell hemoglobin concentration):**

Mean cell hemoglobin concentration.

-**WBC (white blood cell):** DC detection method.

WBC count 1u\l of whole blood.

- **Lymphocyte :** (W.SCR) WBC(small cell ratio):

Ratio % of lymphocyte (small cell) to whole WBC.

-**MXD%** (W.MCR) (WBC middle cell ratio):

 Ratio % Of the summation of basophils , eosinophils and monocyte (middle cells) to whole WBC.

-**Neutrophil** -W. LCR (WBC large cell ratio):

Ratio of neutrophil large cell to whole WBC

-**Lymphocyte** - W.SCC (WBC small cell count):

Absolute count of lymphocyte small cell lu\l of whole cell

-**MXD-** WMCC (WBC middle cell count):

Absolute count of basophil, eosinophil and monocyte(middle cell) in lu\l of whole blood.

-**Neutrophil-** WLCC (WBC large cell count):

Absolute count of neutrophil (large cell) in lu\l of whole blood (Operators ,1999).

 Mean platelet volume (MPV) may be reported with a CBC. It is a calculation of the average size of platelets.

Platelet distribution width (PDW) may also be reported with a CBC. It reflects how uniform platelets are in size (labtestsonline.2012).

Chapter Three

Results

Results

This study was carried out at Khartoum Teaching Hospital from research laboratory and emergency laboratory, during the period from May 2015 to March 2016, to measure CBC in two sysmex machines aiming to compare between this machines. One hundred blood samples were collected in EDTA container (2.5 ml) randomly, CBC estimation was carried by sysmex KX-21n (semi automated method),and the results were analyzed used SPSS computer programme. The results showed that there were insignificant variation in HB, RBC, HCT,MCV, MCH, and RDW-SD , AND RDW-CV in the results of the sysmex in the research laboratory when compared to the results of the sysmex in the emergency laboratory, and there were a significant variation in MCHC when compared between two sysmex ,showed in (tables 3.1). (Table 3.2) showed the results of WBC parameters and there were insignificant variation in all WBC results.

(Table 3.3) showed the results of PLts and there were insignificant variation in all plts results.

Table (3.1) show mean, standard deviation and P.value of Hb, Hct, RBCs count and indices (MCV , MCH , MCHC,) , RDW-SD , AND RDW-CV :

Test	Research laboratory Mean ±SD	Emergency laboratory Mean ±SD	P value
Hb g/dl	12.7 ± 1.9	12.6 ± 1.9	0.6
Hct %	38.5 ± 5.7	39.7 ± 7.1	0.2
RBCs $\times 10^{12}$ /l	4.64 ± 0.72	4.63 ± 0.72	0.9
MCV fl	82.9 ± 8.7	84.8 ± 9.2	0.1
MCH pg	30.1 ± 2.7	27.4 ± 2.8	0.2
MCHC %	33.0 ± 2.7	32.0 ± 2.2	0.004
RDW-SD	44.8 ± 5.5	45.6 ± 6.6	0.4
RDW-CV	14.7 ± 2.3	14 .6 ± 2.3	0.8

Table (3.2): show mean, standard deviation and P.value of (TWBCs count, differential and absolute count):

Test	Research laboratory Mean ±SD	Emergency laboratory Mean ±SD	P value
TWBCs $\times 10^9$/l	6.3 ± 2.2	6.2 ± 2.3	0.8
Lymphocyte %	35.5 ± 12.2	36.5 ± 13.2	0.5
Neutrophil %	53.1 ± 12.9	53.3 ± 13.5	0.9
MXD %	10.9 ± 4.3	10.0 ± 3.6	0.5
Lymphocyte $\times 10^9$/l	2.1 ± 0.77	2.1 ± 0.83	0.8
Neutrophil $\times 10^9$/l	3.5 ± 1.9	3.5 ± 2.0	0.9
MXD $\times 10^9$/l	0.6 ± 0.25	0.6 ± 0.25	0.1

Table (3.3): show mean, standard deviation and P.value of Plt , PDW , MPV , and P-LCR in patients :

Test	Research laboratory Mean ±SD	Emergency laboratory Mean ±SD	P value
Plt $\times 10^9$/l	256 ± 102	264 ± 104	0.6
PDW	12.3 ± 1.8	12.2 ± 1.8	0.8
MPV	9.9 ± 0.9	9.8 ± 1.3	0.6
P-LCR	24.5 ± 6.9	25.5 ± 9.4	0.4

Chapter Four
Discussion, Conclusion and Recommendations

Chapter four

Discussion, Conclusion and Recommendations

4.1 Discussion:

This study involved 100 blood samples which estimated by two different sysmexs kx21n collected from patients who required for routine complete blood count (CBC) investigation in Khartoum teaching hospital from Research laboratory and Emergency laboratory.

The results showed that there was insignificant variation in the mean of hemoglobin, RBC, HCT, MCV, MCH, RDW-SD, RDW-CV ; (p.value 0.6). (p.value 0.9). (p.value 0.2). (p.value 0.1). (p.value 0.2). (p.value 0.4). (p.value 0.8) respectively. And there was significant variation in the mean of MCHC (p.value 0.004).

In the results of WBC parameters showed that there were insignificant variation in all WBC results.

 And in the results of PLts showed that there were insignificant variation in all platelets results.
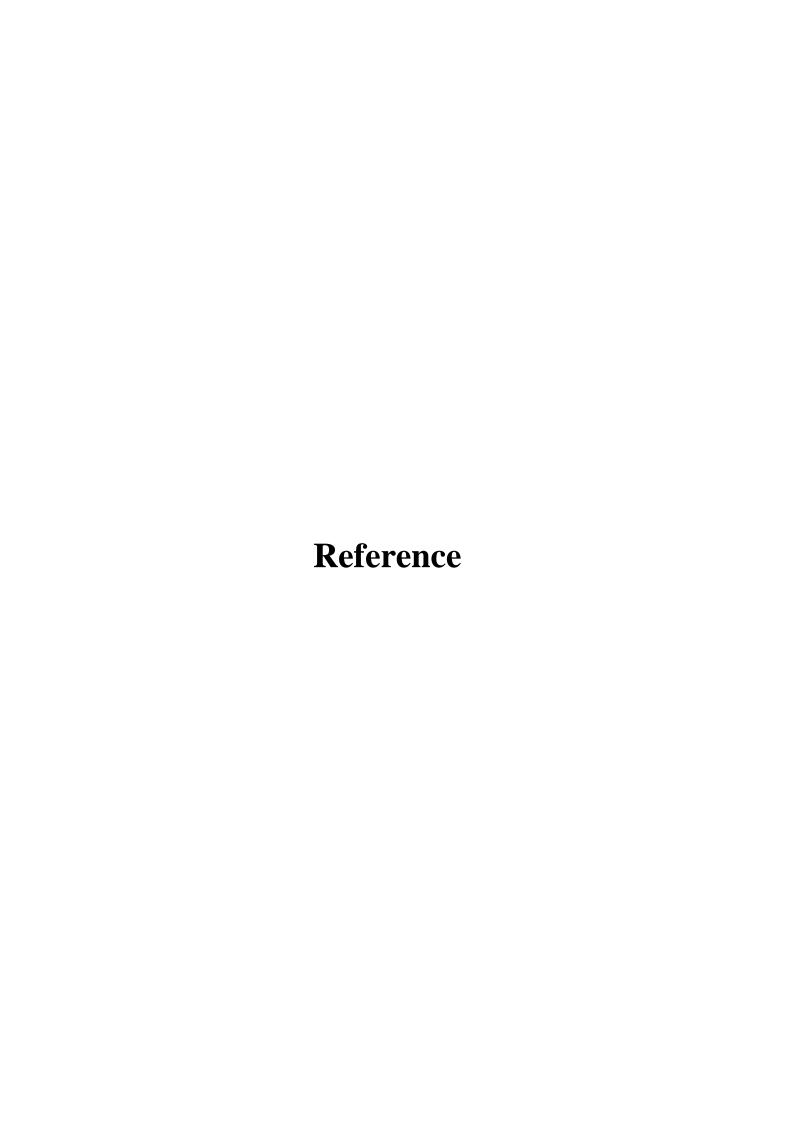
4.2 Conclusion:

This study concluded that after statistic analysis

1- There were no significant difference in the mean of Hb, RBC, HCT, MCV, MCH, RDW-CV, and RDW-SD except in MCHC parameter there was significant variation.

2- There were no significant difference in the mean of TWBC count, differential and absolute count.

3-There were no significant difference in the mean of Plt, PDW, MPV, and P-LCR.

4.3 Recommendation:

The finding of this study recommended that:

- Importance of using well calibrated instruments.

- Estimate the sample as fast as possible, to avoid any change in it.

- K2EDTA is considered the anticoagulant of choice for automated cell counts.

- Training the staff to work competently and economically and use the equipment correct.

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Appendixes

Appendix (1): Sysmex KX-21n