COMPARATIVE ASSESSMENT OF AUTOMATED AND MANUAL WHITE BLOOD CELL COUNTS AT KENYATTA NATIONAL HOSPITAL HAEMATOLOGY LABORATORY

BY

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DECLARATION

I Dr Muturi Charles Kibicho, declare that this work is my original idea and has not been submitted for a degree in any other university to the best of my knowledge

Signed _____ Date _26/3/2012_____

This dissertation has been submitted for the examination with our approval as university supervisors:
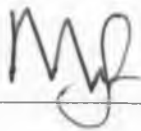
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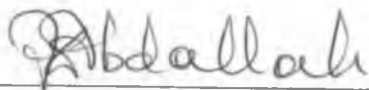
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CONTENTS

LIST OF ABBREVIATIONS

ACD ---------------- Acid-Citrate-Dextrose

ALL ------------------ Acute Lymphocytic Leukaemia

AML --------------- Acute Myeloid Leukaemia

CBC --------------- Complete blood count

CD 1300 --------------CELL DYN 1300 Automated Counter

CD 3200 --------------CELL DYN 3200 Automated Counter

CLL --------------- Chronic Lymphocytic Leukaemia

CML --------------- Chronic Myeloid Leukaemia

CPD --------------- Citrate-Phosphate-Dextrose

CV --------------- Coefficient of variation

EDTA --------------- Ethylene Diamine Tetra Acetic acid

EM ------------------ EDTA anticoagulated Manual Method

ESR --------------- Erythrocyte sedimentation rate

Hct --------------- Haematocrit

Hb -------------- Haemoglobin

HTLV-1-------------- Human T-Lymphocytic Virus type 1

IQC -------------- Internal Quality Control

ISS --------------- Immunosuppression from HIV/AIDS

KNH -------------- Kenyatta National Hospital

MCV --------------- Mean Corpuscular Volume

NC ------------------ Neubauer Chamber Method

NM ------------------ Native Blood Method

NPV --------------- Negative Predictive Value

NRBC --------------- Nucleated Red Blood Cell

PBF -------------- Peripheral blood film

PCV ------------ Packed Cell Volume

PLT --------------- Platelets

PPV --------------- Positive Predictive Value

QC -------------- Quality Control

RBC --------------- Red Blood Cell

SD --------------- Standard Deviation

TNCC --------------- Total Nucleated Cell Count

WBC --------------- White Blood Cell

LIST OF TABLES AND FIGURES

1 ABSTRACT

Background: Assessment of White Blood Cell values is essential in patient management. However many methods currently in use tend to vary, with most centres using different types of automated machines and a few that have no electrical installations may still have to rely on the manual methods. The manual methods are also retained for purposes of calibration of new machines and to occasionally tie-break the results obtained by different methods. It is therefore rational to remain acquainted with and retain the capability of manual methods while being conventional with automated methods

Objective: To compare the WBC values by automated and manual methods

Study Design: A cross-sectional study of automated and manual white blood cell values from March to May 2008.

Setting: Kenyatta National Hospital Haematology department and wards

Measurable Values and Study Outcomes: Age, sex, diagnosis, WBC total counts, WBC differential counts, Hb, PCV, MCV, platelet counts, NRBC, and ESR.

Materials and methods: Blood specimens from the wards sent to the Haematology laboratory for complete (total) blood counts and those which met the inclusion criteria were processed. The white blood cell counts were estimated using cell counters (CD3200 and CD1300), PBF and Neubauer Chamber. ESR was also done by Westergren method. In addition, the patients were traced to the wards for the purposes of obtaining desired clinical information from the patient notes and sample for making the PBF using Native blood. The data was entered into a proforma questionnaire. Comparison of the results by the automated and manual methods was undertaken and data analysis was done using standard statistical tests.

Results: The manual and automated methods had coefficient of variations below 5% for total WBC using control blood. The manual methods showed high accuracy in estimation of WBC compared to automated methods with a correlation coefficient, $r^2 > 0.9$. Manual method with EDTA anti-coagulated blood and native blood were comparable for WBC counts. The TWBC and differentials highly correlated ($R^2 > 0.91$) and the inter-manual (EM and NM) variations were not significant in the mean estimates of these cell populations ($P > 0.05$). The age, sex, Hb, PCV, MCV did not affect the WBC counts, while RBC, platelets and ESR (in females) were found to affect the WBC counts. Manual methods showed a higher sensitivity (85% and 96%), specificity (82% and 67%) and accuracy (42% and 47%) for detection of immature WBC and NRBC respectively when compared to CD3200 sensitivity (83% and 80%), specificity (53% and 33%) and accuracy (35% and 39%) respectively.

Conclusions: Manual methods produced similar results with automated methods for estimation of total WBC, lymphocytes and neutrophils and can be used to compliment automated machines or used in place of automated machine in case of machine downtime with similar accuracy expected. Therefore, manual differential count can be performed when necessary on Native blood. CD3200when compared to manual methods (EM and NM), showed less accuracy in flagging immature white cells and nucleated red cells.

2 INTRODUCTION

The methods used in determining the WBC values are important since the assessment of white blood cell counts play an important role in diagnosis and treatment of patients. These require results that are reliable and accurate. In addition the WBC values provide valuable information about the blood and the bone marrow, which is the blood-forming tissue [1].

The WBC is used for the following purposes: to identify persons who may have inflammatory conditions particularly an infection, acute and chronic illness, blood diseases for example white blood cell disorders such as leukemia, effects of treatments and monitoring of treatment especially to determine the effects of chemotherapy and radiation therapy on blood cells [1-6].

Usually the values estimated are the total counts and differential of the various components. A differential determines the proportion of each of the five types of mature white blood cells [1].The relevance of these can be viewed in the following aspects, an elevated WBC count occurs in infection, allergy, systemic illness, inflammation, tissue injury, and leukemia. A low WBC count may occur in some viral infections, immunodeficiency states, and bone marrow failure [1,2]. The differentials will reveal which WBC types are involved and affected. For example, an elevated WBC count with an absolute increase in lymphocytes having an atypical appearance is most often caused by infectious mononucleosis. The differential will also identify immature WBC that may be reactive (a response to acute infection) or the result of a leukemia [1, 2].

The methods used to obtain manual WBC values include total counts estimation by both Neubauer chamber and peripheral blood film. The latter method is also used for the manual

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differential counts. The automated methods used mainly depend on the manufacturer but operate based on either one of two principles; impedance and optical technology. They can assess both total and differential or total white cell counts only. In recent times, many centres have adopted the automated methods due to a variety of reasons.

It is, however, well to remember that despite the usefulness and, indeed, essential role of automation in the modern diagnostic laboratory, the haematologist cannot function on automated machines alone. In addition, there are settings that may not adopt many of the automated methods. Despite the automated differential leucocyte count and automated procedures for determining blood cell parameters, microscopy and morphological expertise remain the basis of diagnostic haematology [1, 6]. There will always be an unexpected diagnostic situation which will require human skill for interpretation. Thus, while the automated system may be getting increasingly independent of human supervision, it is certainly well to remember that haematology has always been and remains largely a clinical art. It is vital that distinction be made between having dependable machines and becoming machine dependent. Finding the right path is perhaps the greatest problem in automatic analysis in haematology. This requires a closer collaboration between the manufacturers and the haematologists so that they can share their respective skills for the development of machines which are both relevant and reliable [6]. It is against this background that this study is being undertaken.

3 LITERATURE REVIEW

Historically examination under microscope of good quality blood smears has been the best way to estimate the number of leukocytes or white blood cells; for leukocyte differentiation; to study the morphology of erythrocytes or red blood cells; to characterize the morphology of lymphocytes and; to calculate the number and morphology of platelets [1, 5, 7 - 9]. From the last 20 years automated systems for leukocyte recognition are currently available in the market and are used in clinical laboratory routines. These generally rely on flow cytometry and impedance techniques whereby a blood sample flows through a detector and is then discarded. In addition these devices reach the limited aims of identifying normally circulating leukocytes and of flagging abnormal circulating cells. Thus examination of stained peripheral blood smears remains necessary for detecting and classifying abnormal cells [1, 5] particularly to study the morphology of lymphocytes which is regarded as the principle basis for the identification and discrimination among the chronic lymphoproliferative disorders [5].

When the electronic WBC count is abnormal or a cell population is flagged, meaning that one or more of the results is atypical, a manual differential is performed. In that case, a wedge smear is prepared. This is done by placing a drop of blood on a glass slide, and using a second slide to pull the blood over the first slide's surface. The smear is air dried, then stained with Wright stain and examined under a microscope using oil immersion (1000x magnification). One hundred white cells are counted and identified either as neutrophils, lymphocytes, monocytes, eosinophils, or basophils based on the shape and appearance of the nucleus, the color of cytoplasm, and the presence and color of granules. The purpose is to determine if these cells are present in a normal distribution, or if one cell type is increased or decreased. Any atypical or immature cells also are counted [1].

In addition to determining the percentage of each mature white blood cell, the following tests are performed as part of the differential:

Evaluation of RBC morphology is performed. This includes grading of the variation in RBC size (anisocytosis) and shape (poikilocytosis); reporting the type and number of any abnormal RBCs such as target cells, sickle cells, stippled cells, etc.; reporting the presence of immature RBCs (polychromasia); and counting the number of nucleated RBCs per 100 WBCs [1].

An estimate of the WBC count is made and compared to the automated or chamber WBC count. An estimate of the platelet count is made and compared to the automated or chamber platelet count. Abnormal platelets such as clumped platelets or excessively large platelets are noted on the report [10 – 12]. Any immature white blood cells are included in the differential count of 100 cells, and any inclusions or abnormalities of the WBCs are reported.

Sources of error in manual WBC counting are largely due to variance in the dilution of the sample and the distribution of cells in the chamber, and the small number of WBCs that are counted. For electronic WBC counts and differentials, interference may be caused by small fibrin clots, nucleated RBCs, platelet clumping and unlysed RBCs. Immature WBCs and nucleated RBCs may cause interference with the automated differential count. Automated cell counters may not be acceptable for counting white blood cells in other body fluids especially when the number of WBCs is less than 1000/μL or when other nucleated cell types are present [13].

NRBCs have the potential to interfere with WBC determination because they contain nuclei. When exposed to the lyse reagent they form a nuclear particle similar to leucocytes. In majority of cases, NRBCs are present in such low numbers that they do not affect the WBC count/ differential. Band cells react to the lyse reagent just as mature neutrophils and are incorporated into the granulocyte peak. They do not form a$_6$separate population. Left shifts are typically

accompanied by an elevated total WBC count which should further be characterised by a PBF evaluation. The shift will be noted at that time [14 – 18, 46].

One effect that can be a problem for all cell counters is turbulent flow around the aperture and sensing zone. If flow is not laminar, the same cell may be counted several times as it tumbles in this zone, resulting in an inaccurate cell count or size measurement. It could indicate the wrong size because the tumbling cell might only partially enter the sensing zone, so it would appear to be smaller than it actually is. To ensure laminar flow, some machines, for example Beckman Coulter uses a method called sweep flow. Sweep flow is a steady stream of diluent flowing behind the RBC aperture during the count time and prevents tumbling cells.

Another problem encountered with impedance machines crops up when the cell passes through the aperture near its edge, or at an angle not parallel to the center axis. As it moves, the cell creates atypical pulses. A process called pulse editing was developed to eliminate atypical pulses from the analysis because they distort the true cell size.

A pandemic problem among most analyzers performing differential white cell counts is an inability to distinguish between small lymphoid blasts, circulating small lymphoma cells, and normal lymphocytes. Thus, flags may not be displayed even in the presence of abnormal lymphoid populations. It has been suggested that automated laboratories set policies for reviewing sentinel slides in patients with unexpected absolute lymphocytosis to avoid missing malignancies in the lymphoid system [19]

For a WBC differential there are two major types of errors encountered; due to random distribution of cells in diluted specimens which is inherent whether cell counting is by automated or manual methods, due to cell identification [10].The random distribution error depends on percentage of given cells and the total number of cells counted for the WBC differential. The lower the percentage of cells, the higher the range of error. The range of error of a normal

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total WBC count is about 20% by manual count and 1% by automation. At very high or very low WBC counts, the accuracy compared with the automated counter may vary. For a manual WBC differential, generally 100 white cells are counted compared to the 8000 cells counted by the automated machine. If the instrument can identify the cells properly the range of error by the automated method is generally lower than the manual differential [1, 20]. However on rare occasions, presence of cold agglutinins / cryoglobulins in the blood can result in falsely high WBC counts because the machine mistakenly counts the aggregated red cells or proteins as WBCs [1].

With films made on glass slides, it is generally assumed that the distribution of leucocytes is random. For an accurate differential count on slides, the film must not be too thin and the tail of the film should be smooth. To achieve this, the film should be made with a rapid movement using a smooth glass spreader. This should result in a film in which there is some overlap of the red cells, diminishing to separation near the tail, and in which the leucocytes in the body of the film are not badly shrunken. If the film is made too thinly, or if a rough-edged spreader is used, many of the leucocytes (50%) accumulate at the edges and in the tail. This is also true of monocytes while lymphocytes will tend to predominate in the middle of the film. No system of counting will compensate for the gross irregularities in distribution in a badly made film. It is a waste of time to attempt a differential count on such a film and, if this is attempted, futile to count only cells in the centre of the film, where lymphocytes probably predominate, and to neglect altogether the tail, where most of the neutrophils lie. A well made film is one in which many leucocytes are present in the body of the film and there is no great accumulation at the tail

In manual counts the observed differential depends not only on artificial differences in distribution due to the process of spreading, but also on 'random' distribution; this later effect is naturally reduced by counting a large number of cells. Theoretical considerations and

practical studies have shown that with a true neutrophil proportion of 50% the range (+/- 2SD) within which 95% of the counts will fall if 200 cells counted is of the order of about +/- 7%, ie 43- 57% neutrophils; and if 100 cells are counted +/- 10%, ie 40 - 60% neutrophils. If 500 cells are counted the range would be reduced to 4.4%, ie 45.6 – 54.4% neutrophils. With percentages above 50, the range is proportionately less, and below 50, greater than the above figures [1]. 'Confidence curves' based on calculations of standard deviation and assuming a perfect distribution, have been published and from these one can find the range within which lies the true value (95% probability) corresponding with any observed percentage count. The usefulness of these curves is limited because they can only be applied to well spread films. It is futile to analyse statistical results obtained from a badly spread film [1].

The improved precision and speed of the automated differential count over the 100-cell manual differential is well documented. The imprecision and inaccuracy of the manual differentials results from the limited number of cells viewed and maldistribution of cells in wedge preparations. The manual differential is the accepted reference method for evaluating an automated differential but poses problems in evaluating the automated monocyte and basophil results. The correlation between the automated and manual differential for neutrophils, lymphocytes, and eosinophils is described as good, but because of low numbers in manual counts, there is poor agreement between the manual and automated basophil and monocyte counts [21].

Most instruments use impedance and optical technologies to measure the total WBC count. Predictably, both technologies have their limitations. In impedance counting, both high and lower range accuracy may be impaired. Counts may also be inaccurate in the presence of circulating nucleated red cells. Optical methods, however, enjoy substantially better high and low end accuracy than impedance techniques, but they may be impaired in the presence of fragile WBCs

and lyse-resistant red cells [22].

In certain situations the morphology of particular leucocytes may be altered as to cause their misinterpretation either by manual or automated methods.

In Alder-Reilly anomaly, the granules are very large, discrete, stain deep red and may obscure the nucleus. There is a possibility of such cells being labelled as eosinophils during manual counts. Other leucocytes, including some lymphocytes, also show the abnormal granules. Some of the lymphocytes may end up being counted as granulocytes especially by an inexperienced person [1].

Small numbers of dead or dying cells may normally be found in the blood, especially when there is an infection. They may also develop in normal blood in vitro after standing for 12-18 hours (storage artefact), even if kept at 4°C. These cells have a round, dense, featureless nuclei and their cytoplasm tends to be dark pink. It is important not to confuse these apoptotic/pyknotic cells with normoblasts during manual counts [1,43].

It is occasionally difficult to distinguish monocytes from the large activated T lymphocytes produced in infectious mononucleosis or from circulating high-grade lymphoma cells [1,4]. The presence of smudge cells (crushed cells during spreading of the blood film due to their increased fragility) in leukaemia may present another challenge since the manual count obtained may reveal a lymphopenia especially if performed by an inexperienced person.

Other potential causes of error include mistaking dirt or clumped red cell debris for leucocytes, and the clumping of leucocytes. The latter, usually several leucocytes stuck to debris, seems to occur particularly in heparinized blood, especially when the concentration of heparin is high. The clumps are most frequently seen in blood which has been allowed to stand for several

hours before being diluted. Once the blood is diluted, it seems safe to leave the suspension for several hours before undertaking the count [1].

Platelet aggregation [10 – 12] or partial clotting of the blood specimen especially if EDTA is used as an anticoagulant can lead to their inclusion in the differential count. The same problem can be encountered with platelet satellitism around RBCs [10 – 12].

Fully automated multi-channel instruments perform WBCs by either impedance or light scattering technology or both. Residual particles in a diluted blood sample are counted after red cell lysis [2, 23, 24] or in the case of some light scattering instruments, after the red cells have been rendered transparent [1, 25 – 27, 38, 40, 52]. Thresholds are set to exclude normal platelets from the count, although giant platelets are included. Some or all of any nucleated red cells present are usually included, so that when nucleated red cells are present the count approximates more to the TNCC than to the WBC [1, 43]. Hence correction of the WBC count should always be done when the NRBC percentage is high (see Appendix IIIA on corrected WBC counts formula). However care should be taken to differentiate small lymphocytes from NRBC.

Factitiously low automated WBCs occasionally occur as a consequence of white cell agglutination. Factitiously high counts are more common and usually result from failure of lysis of red cells; with certain instruments, this may occur with cells of neonates or be consequent on uraemia or on the presence of an abnormal haemoglobin such as haemoglobin S or haemoglobin C [1]. Moreover, it has also been shown that white cell counts can be influenced by factors such as age, sex, obesity, smoking status and ethnic differences [48, 49, 50].

When the cell morphology is abnormal, automated instruments may not recognize white cells effectively because once the machine is set up to count cells of a particular size, it matters little what is actually being counted and in this [11] scenario a technologist's expertise becomes an

important factor in providing an accurate cell count. Understanding the methodology of differential cell counting [30 – 34, 37 - 40] and instrument flagging is important in deciding which films should be reviewed based on instrument parameters. For laboratories with low testing volume, it may be appropriate to review some or all films even when the criteria for release of the automated counts are met to assist with the assurance of ongoing technologist competency in manual differential counting in this setting may serve as a check on manual differential performance accuracy. Particularly in the setting of fragile cells such as lymphocytes the manual counts may underestimate the lymphocyte number and therefore spuriously increase the relative number of neutrophils [35, 36].

Although there is no clinical rationale for reflexively performing manual WBC differential counts based solely upon instrument flags, prior to a manual triage step, it is important to distinguish between validation and medication interpretation in the clinical haematology. For example although manual validation of absolute lymphocytosis may not require performance of a manual differential count it still may be necessary to issue a medical interpretation of such a case by a haematologist regarding the nature of the circulating lymphocytes and the likelihood of chronic lymphocytic leukaemia or other leukaemic lymphoproliferative disorder [1, 35, 36, 47].

In one German study, the CELL DYN 3200 was evaluated in a tertiary care hospital laboratory with regard to precision and accuracy of the 5-part white blood cell differential provided and clinical sensitivity and specificity of the instrument flagging system. Short- and long-term precision was fully satisfactory in normal and abnormal non flagged specimens. CELL DYN 3200 values corresponded closely with those of reference microscopic 400-cell differentials: in normal samples, average bias did not exceed 1% for any cell type. Moreover, in abnormal non flagged specimens, no case with gross discrepancy between CELL DYN 3200 and microscopy results was observed; linear regression coefficients were 0.96, 0.95, 0.68, 0.95, and 0.57 for neutrophil, lymphocyte, monocyte, eosinophil, and basophil percentages, respectively. The

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instrument diagnostic efficiency was 88.5% for morphologic abnormalities only and 90.6% if both morphologic and distributional abnormalities were considered. False - negative results were largely confined to some cases with moderate elevation of immature granulocytes and band cells [38]. The differential leucocyte counts provided by the CD3200 compared well with microscopic 400-cell differentiation. With regard to neutrophil, lymphocyte and eosinophil counts discrepancies exceeding the range given by the precision of microscopic examination were restricted to a small minority of samples and never were of major clinical importance. Such discrepancies were somewhat more frequently encountered for monocyte counts. This phenomenon is caused by the difficulties in identification of this cell type by both flow cytometry and microscopic differentiation and can be observed regularly in evaluation studies [39, 40, 44]

In one China study, the objectives of which were to research on the accuracy of automated hematology analyzers of various types from different manufacturers and to observe the deviation among these instruments was undertaken. Fresh anticoagulated blood from healthy donors on 115 hematology analyzers in 114 different hospitals were determined. The maximum coefficients of variation (CVs. %) among instruments of three main manufacturers (Sysmex, Beckman Coulter and Abbott) of red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), white blood cell count (WBC) and platelet count (Plt) were 3.2%, 3.8%, 3.6%, 9.3% and 10.8%, respectively. The maximum deviations among these parameters of different instruments were 0.74%, 1.65%, 5.45%, 7.06% and 18.55%, respectively.it was noted that by improving laboratory quality management, the results of hematology analyzer determination may be more reliable than manual methods. The difference among various manufacturers was very small about RBC, Hgb, Hct, WBC and Plt such that the results from all kinds of instruments will tend to be comparable [45].

Unfortunately however, many labs still replace 13 automated counts with manual differential

leukocyte counts solely based upon the presence of instrument flags. Such an approach raises problems. For instance, an instrument flag may be issued for suspicion of cell types not actually in the blood sample (false positives), resulting in the replacement of a perfectly valid automated differential count by the less accurate and less precise manual differential count. Indeed, previous studies have confirmed that the flagging systems in hematology analyzers tend toward false-positive flagging, with false-negative rates that are considerably lower than those seen by manual blood smear review. Furthermore, manual differential counts are often performed due to the presence of solely quantitative abnormalities, even when individual cell identification is not in question. For instance, markedly elevated absolute lymphocyte counts in patients with known chronic lymphocytic leukemia may lead to manual differential counts, when the only real reason for manual review is confirmation that the cells in question are in fact lymphocytes and not another abnormal cell type such as blasts or large lymphoma cells [1].

4 BACKGROUND AND RATIONALE

It may not be obvious to many that several times the haematology departments even in the KNH have to resort to manual methods when the rather expensive automated machines fail, are newly installed or the reagents are a problem. Those in the training are well aware that the first lessons in blood cell counting are and have been manual methods using the Neubauer chamber. In fact every haematology laboratory particularly those in the large teaching and referral centres should have methods to be used to calibrate newly installed machines and also to restore discrepancies obtained by different cell counters. That is the reason why there are established methods for all cells and parameters in various mature laboratories [51]. All automated blood cell counters measure a variety of values and these must be determined initially from the manufacturer and considered individually when planning an evaluation. Particular attention must be paid to the use of a calibrator which may be single- or multiple-value material [27, 52].

Therefore in spite of sophistication and the ever changing improvements of automated haematology analyzers there are still good rationale for retaining not only the concepts and practice of manual cell counting techniques and methods but also perfect and understand the indications, the variance with the automated methods of the manual methods. Any setting/establishment which does not have ability to perform manual cell counting is tied strongly to very laborious often unsuccessful techniques of sorting out trouble shooters in automated machine. Indeed such systems cannot calibrate, standardize and internally control the wayward machines. The system of automation must incorporate the necessary technological background needed for running and supporting the automation. Such include electrical power installations, good computer working systems and elaborate system requiring a working chain of highly qualified personnel. The manual system requires very basic apparatus and highly motivated well experienced members of staff. The manual system can be applied even in the remotest of systems and settings e.g. in refugee camps and screening epidemics where there is

no electrical power installation or permanent infrastructure in terms of buildings or running water. In teaching institutions, all those taking courses in haematology are still required to have a working knowledge of manual methods. It will certainly be sometime before the manual cell counting becomes obsolete. To teach and apply manual methods require that it be assessed for accuracy. It is the intention of this study to subject this through an investigation.

Variations in automated and manual WBC counts are expected however no local study has been done to define the level of concordance or otherwise. The results of this study will enable us find out more about the level of variations and their attributes (causes).The study will enable us in adjustment and narrowing down the level of inconsistencies by improving on factors causing them while maintaining any similarities. The results generated shall also add value to the existing sparse literature on automated and manual WBC parameters.

HYPOTHESIS

There are no differences in white blood cell values as estimated by automated and manual methods

OBJECTIVES OF THE STUDY
General objective

To compare the white blood cell values by automated and manual methods.

Specific objectives

1. To determine the coefficient of variation in WBC values between automated and manual methods

2. To compare the variations of the white cell values with age, diagnosis, Hb, PCV, MCV, platelet counts, nucleated red cells/ 100 WBC (RBC/100WBC) and ESR.

3. To compare the WBC values by the two manual methods on the native and anti-coagulated blood

4. To compare the estimated WBC values on the blood films with the automated values

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5 MATERIALS AND METHODS

Study, design: This was a cross sectional study.

Study area: Kenyatta National Hospital Haematology Department and wards. Kenyatta National Hospital is the largest referral hospital in Kenya receiving a large number of blood specimens from an equally large population of patients with diverse disease conditions. The hospital has in addition, the availability of trained/ skilled staff.

Identification and recruitment of study cases

To get the data the principal investigator perused through the register in the haematology department at about 1pm and 3pm every day. This entailed getting every sixth recorded sample of blood sent for complete (total) blood cell counts, out of a daily total average of 240specimens received at the laboratory. This was necessary to avoid the bias of having samples from the same ward and with similar disease conditions. The selected specimens were then identified using the request forms and the laboratory numbers. The specimens were then ascertained for the requirements as per the inclusion criteria. The principal investigator then used the addresses obtained to trace the individual cases to the wards. In the ward he introduced himself and the purpose of the study to the senior house officers and other members of staff. These members of staff identified the potential patients (cases). The patient was then approached and the purpose of the study explained to him/her. After this, consent was sought and patient recruited. The information regarding the study was discussed so as to get the patient participate in the study. Informed consent was obtained from the patients for the purposes of perusing the

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personal records and drawing blood for PBFs using native blood and to obtain additional specimen in cases where the first was insufficient. After this the records of the patients were reviewed for the required information. All relevant data as stipulated in the proforma questionnaire was extracted from the patient's records including request form, patient case notes and also the patient where some details are not found in the records. Native blood specimen was then taken and a PBF was made by the bedside. The minimal information required in the request form, included the name of the patient, age, sex, ward, time of specimen collection and the working diagnosis.

Specimen collection and transport

The required 4ml of venous blood was collected using a G21/23needle by the principal investigator or phlebotomist or clinician in the wards. Blood was put in appropriate bottle with EDTA (ethylene diamine tetra acetic acid) after which it was transported to the laboratory as soon as possible to enable tests be performed within 3 hours of specimen collection. While at the bedside blood films using native blood were made at the patient's bedside and allowed to air dry before being taken to the laboratory for staining and evaluation. This was done by the principal investigator with the assistance of the ward phlebotomist or laboratory technologists.

Laboratory procedures

The anti-coagulated specimen was divided using a pipette, into three equal portions/aliquots by the principal investigator into aliquot containers. Each of the portions was assigned a different code such that for every specimen had parts A,B,C,D,E representing CELLDYN 1300, CELLDYN 3200, manual EDTA, Neubauer chamber counts and ESR respectively.

Of the three portions, one was run in the first machine- CELL DYN 1300, while the other portion was run in the second machine- CELL DYN 3200. The latter machine was used as the

gold standard for the automated counts. The choice was made, based on the information provided by the haematology technologist-in-charge regarding the three machines available at KNH of which CD3200 was more reliable and being used for IQC within the laboratory. All the procedures were done by the principal investigator with the assistance of the laboratory technologists and the results obtained were counterchecked by the study supervisors. In order to avoid bias while performing the counts, the results from the automated method were only retrieved after the manual counts had been concluded.

The third blood sample/aliquot was used to make PBF for manual counts for total and differential counts. The slides for PBF evaluation using both anti-coagulated and native blood were then be stained by Romanowsky (MGG) stain.

One sample of the anti-coagulated blood was placed in the Neubaeur chamber (see appendix IIIA) for enumeration and calculation of the TWBC counts. This was done by the principal investigator with the assistance of a technologist. The PBFs from both native sample and anti-coagulated blood was used for total WBC estimation and differential counts (Appendix IIB). Examination of the PBF from both the native blood and anti-coagulated blood was performed by the principal investigator and reviewed with supervisors. In the specimens where NRBC were present, correction was done to obtain the corrected values (Appendix IIIA). This was done by the principal investigator and then counterchecked by the study supervisors. The principal investigator performed the ESR in the presence of the assisting laboratory technologist. (Appendix IIIB)

The last step involved reconciliation of the results obtained by the two machine counts and those of manual methods. This was undertaken by the principal investigator and counterchecked by the study supervisors.

Selection criteria;

- Patients who consented to the study
- Freshly collected specimens (not more than 3hr since collection) and preserved specimens (at 4° C for not longer than 24hr since collection)
- Availability of samples, native blood films and desired patient details as per the questionnaire

Exclusion criteria;

- Poorly processed slides for manual WBC assessment
- Specimens that were less than 2ml
- Clotted samples
- Samples whose requests did not have complete information about the patients
- Patients who did not give consent

Reporting and diagnosis

The results were reported by the use of standard reporting methods used in the Haematology Unit at the KNH(Appendix III), then entered into a proforma questionnaire(Appendix I).

Quality control

The standard operating procedures of KNH Haematology laboratory were adhered to, with regards to processing and handling of specimens for blood counts, blood films and ESR In addition further steps were undertaken for the purposes of the study cases. This was necessary as KNH is the largest national and referral hospital acting as the reference centre/pacemaker for medical activities in Kenya. Also, it is on this basis that investigations and teaching is done at the Haematology Unit.

Prevention of pre-analytical errors

Specimens were collected in the right bottles, adequate amount (4ml), properly mixed, and where applicable with the appropriate anticoagulant. Specimens were taken in the presence of trained phlebotomists stationed in the wards. The standard operating procedure for phlebotomy and other blood taking procedures strictly followed positive identification of the specimens and ensuring that the blood samples were properly labeled so as to avoid misidentification errors. Analysis was carried out within the required time

Prevention of analytical errors

The laboratory technologist and principal investigator ensured that proper maintenance and calibration of the machines used in the automated counts had been carried out as per manufacturer's recommendations. Stabilized blood using CPD and passed through a blood infusion set to remove any clot was used as the material for IQC. The material comprised a normal control sample. The controls were used to validate both the automated and manual methods. The controls were included into each run of the machines and were also used in the manual methods (PBF, Neubauer Chamber and ESR). The staining of the PBF slides was done by an experienced technologist using standard procedure (Appendix 3B). The slides were then examined at low power (x 10)before assessment at x 100 with oil immersion. The TWBC was calculated using the formula in Appendix IIIB and the battlement pathway/ method was used to assess and count the white cell sub population by the principal investigator. The slides were then reviewed by one of the two supervisors and where significant variations in the counts were noted the second supervisor acted as a blinded reviewer. The results obtained were then discussed and a consensus agreed upon.

Data Analysis

Data was analyzed using Instat™ for window version 3.02 (GraphPad software Inc.). Data was presented in mean ±SD. Analysis of all the demographic data was be performed using χ2 . Similarities and differences between manual and CELLYN 1300, manual and CELLDYN 3200, manual (native blood) and manual (EDTA), CELLDYN1300 and CELLDYN3200 were evaluated using student t-test (two sided). Analysis of variance was used to compare means of WBC counts among all the methods. Pearson correlation coefficient was used to correlate the counts between methods. The coefficient of variation was calculated for the control samples to validate the results. Data was presented in tables and figures.

Sample size

Fisher & Van belle) formula for sample size calculation

Primary outcome variable =presence of variation in counts by different methods

Best guess of expected percentage (proportion) variation = 20%

Desired width of 95% confidence interval

Using formula $\quad n = \dfrac{Z^2 p(1-p)}{\partial^2}$

Where;

n= required sample size

p= expected proportion

∂=The required precision (0.05)

Z= the value corresponding to 95% confidence interval

Substituting the above formula we get:

N= (1.96) 2x 20%(1-20%)

0.052

= 245 specimens.

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Ethical Issues

1) Authority to carry out the study was sought and obtained from the KNH Ethical and Research Committee

2) The study was carried out upon approval

3) Informed consent was sought from patients or the parents/guardian for patients too ill to give consent or those below 18 years of age. Consent was necessary if repeat specimens had to be taken from the patients and also because smears had to be made using native blood (blood not in anticoagulant)

4) Usual care and evaluation procedures were facilitated for study patients and results of investigations were communicated to the usual health care providers to facilitate improved care of patients

5) There was no extra cost incurred by the patients

6 RESULTS

In this study conducted between March and May 2008, that compared the performance of automated(CD3200 and CD1300) and manual methods(EM, NM and NC) in evaluation of white blood cell counts, a total of 246 patients of different; ages, sex and disease states were enrolled and investigated. The data were initially categorized by age, sex, the working diagnosis and total blood picture was determined.

General characteristics of the study participants

The characteristics of the studied cases were as stated below.

Age; the youngest patient was a two days old infant while the oldest was a 68 year old man, the mean age was 26.7 years (median = 28 years; mode 38 years). Majority of the participants were aged between 20 and 68 years, 61% (151/246) (see table 1).

Sex; General proportions were; females, 50.8% (125/246) and males 49.2% (121/246) respectively with a male: female ratio of 0.9:1, however the difference between among the male and female participants in different age groups was not statistically significant (χ^2=14.592, df=5, ρ=0.3; Table 1)

Table 1: Number of patients their age groups and sex

Age (years)	Number investigated		
	Males n=121	Females n=125	Total n=246
<1 year	12 (40)	18 (60)	30 (12)
1-5	11 (58)	8 (42)	19 (8)
6-10	13 (76)	4 (24)	17 (7)
11-15	12 (80)	3 (20)	15 (6)

16-20	5 (36)	9 (64)	14 (6)
>20	67 (45)	83 (55)	151 (61)

Comparisons Between Manual And Automated Methods

Three manual methods and two automated methods were used for estimation of hematological parameters. Cell Dyn 3200 (CD3200) and Cell dyn 1300 (CD1300) were used for the automated method with the latter being able to give the TWBC and not WBC differential counts. For manual method, blood films were prepared from blood drawn immediately without adding in anticoagulant (NM) and from blood anticoagulated using EDTA (EM). Neubeur chamber (NC) was also used for manual total WBC count. CD3200 and EM were used as the main comparison methods for automated and manual techniques respectively.

Table 2: Mean and SD as estimated by different methods

Paramter	Test samples (n=246)						Control samples (n=23)		
	CD3200 Mean ±SD	NM Mean±SD	EM Mean±SD	CD1300 Mean±SD	NC Mean±SD	p	CD3200 Mean±SD	Manual Mean±SD	
Total WBC x 10^9/l	9.3±6	8.35±5	8.6±5.4	8.43±5	8.6±5.3	>0.05	5.28±0.18	5.17±0.2	>0.05
Neutrophilsx 10^9/l	5.4±4.4	4.8±3.7	5.0±3.8			>0.05	3.3±0.1	3.2±0.1	>0.05
Lymphocytes x 10^9/l	2.1±1.3	2.4±1.3	2.5±1.4			<0.05	1.8±0.07	1.8±0.07	>0.05
Monocytes x 10^9/l	0.66±0.76	0.3±0.3	0.23±0.3			<0.05	0.17±0.04	0.1±0.03	<0.05
Eosinophils x 10^9/l	0.34±1.3	0.2±0.5	0.14±0.5			>0.05	0.03±0.01	0.1±0.03	<0.05
Basophils x 10^9/l	1.4±2.1					>0.05			
Platelets x 109/l	327±136	328±120	330±119	316±126		>0.05			
RBC x 10^{12}/l	4.5±1.0			4.3±1.1		>0.05			
Hb (g/dl)	12.3±3.0			12.2±3.3		>0.05			
PCV (%)	37.4±9.1			35.9±9.2		>0.05			
MCV (fl)	84.9±9.4			84.4±10.3		>0.05			

Table 2 above shows the means and SD of the haematological parameters measured in participant's and controls samples using different methods. There was a significant variation in estimation of lymphocytes and monocytes in patient samples by CD3200, CD1300, NM, EM and NC and in monocytes and eosinophils using CD3200 and manual method on control blood

($p<0.05$). There was no significant difference in estimation of platelets, red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV) and mean cell volume (MCV) as shown in table 2 ($p>0.05$).

Results of validation of the methods using control samples

In this study, twenty three normal control samples (whole blood) at one sample per day were concomitantly analyzed for WBC during the period that the study was carried out. Levey-Jennings Quality control charts[54] were then generated to test the validity of the Automated and manual WBC QC count, mean and SD were used to prepare the QC charts.

The ranges for the WBC counts by automated method were, 4.98 to 5.52 $\times 10^9$/l (total WBC), 3.1 to 3.5 $\times 10^9$/l (neutrophils), 1.6 to 1.75 $\times 10^9$/l (lymphocytes), 0.1 to 0.3 $\times 10^9$/l (monocytes), 0.01 to 0.05 $\times 10^9$/l (eosinophils) and 0 to 0.03 $\times 10^9$/l (basophils). Ranges for WBC counts using manual methods was as follows; 4.8 to 5.52 $\times 10^9$/l, 3.0 to 3.4 $\times 10^9$/l, 1.6 to 1.9 $\times 10^9$/l, 0.051 to 0.2 $\times 10^9$/l, 0 to 0.12 $\times 10^9$/l and 0 for total WBC, neutrophils, lymphocytes, monocytes, eosinophils and basophils respectively.

The results showed that WBC counts for the control samples were within the expected reference range when CD3200 and manual counts were done. Using the recommended Westgard's 3SD rule [55] (i.e reject the samples in the run where the control exceeds 3SDs from the mean of the QC values), all the QC samples fell within 2SD. Therefore all the results using CD3200 and Manual count were valid.
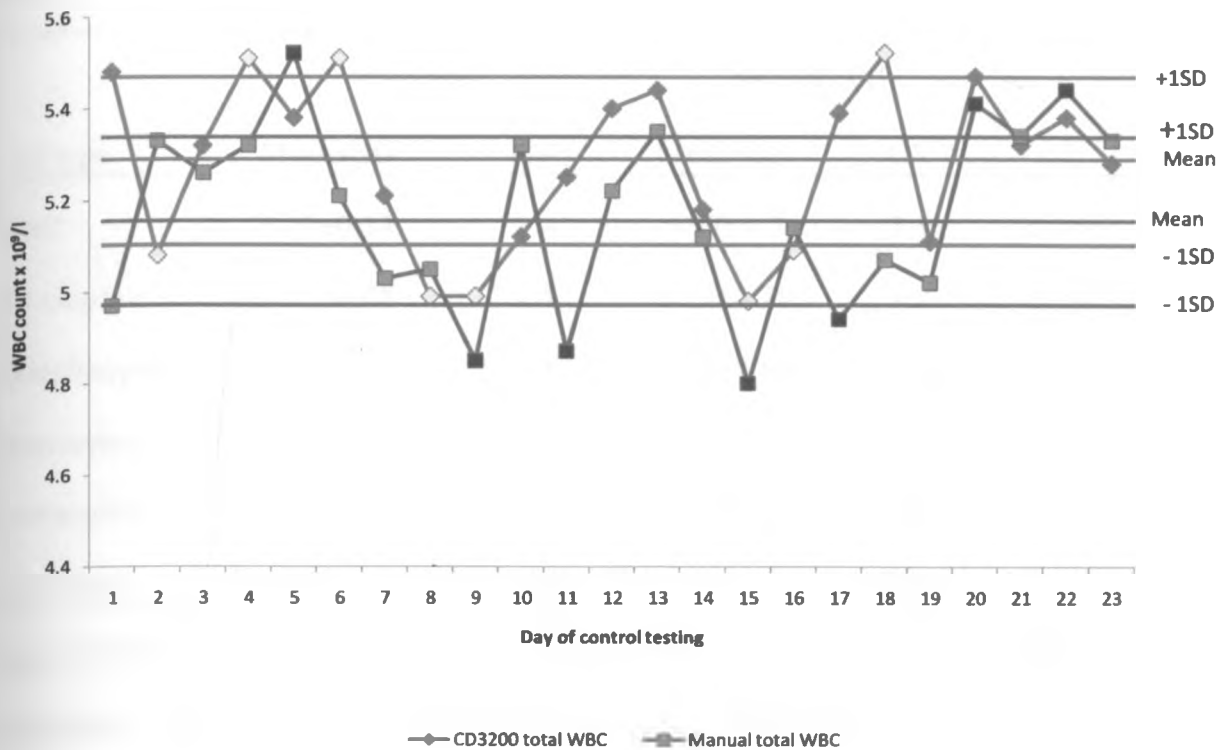
Figure 1: Levey-Jennings QC chart prepared from the WBC count. Blue lines are for WBC counts using CD3200 automated hematology analyzer while the green line is for WBC counts using manual method. QC value above ±1SD is shown by red data points (manual method) or in yellow (CD3200)

Seven out of the 23 samples that were run fell outside 1SD for manual method compared to 8 out of 23 for CD3200 method. The manual method was more precise using 1SD rule [55]; however, results for both methods were within 2SD and hence validating the whole data.

Means, Standard Deviation and Coefficient of Variation (CV) of quality control samples

The mean control values, SD and CVs of the QC sample results using CD3200 and manual counts were calculated. For both techniques, the CVs for total WBC count, neutrophils and lymphocytes were below 5%. The CVs for eosinophils, monocytes by both methods and basophils by CD3200 were relatively high (>20%). There was no significant difference in the mean±SD of total WBC count and neutrophils (p>0.05) using both methods. A significant variation was however seen in the percentage estimates of lymphocytes, monocytes and eosinophils between both methods (table 3).

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Table 3: CV values of QC samples using manual and CD3200 WBC counts

Cell type	CD3200		MANUAL		
	Mean±SD	CV	Mean±SD	CV	
WBC	5.28±0.18	3.4	5.17±0.2	3.9	0.0563
Neutrophils	62.6±0.68	1.1	62.5±0.79	1.2	0.6477
Lymphocytes	33.5±0.71	2.1	34.2±0.69	2.01	0.0015
Monocytes	3.2±0.72	22.4	2.2±0.62	27.4	<0.0001
Eosinophils	0.47±0.19	40.5	1±0.52	52	<0.0001
Basophoils	0.23±0.14	60.8	0	ND	ND

Figure 2 below shows the comparative differences in the CV values of percentage WBC sub populations. There was no significant variation in the CVs between methods.
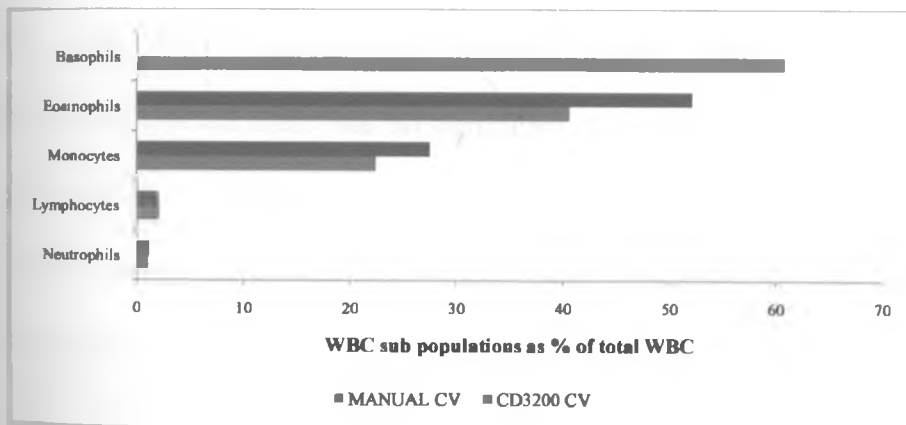
Figure 2: Differences in CVs between the methods

There was no significant variation in the CVs between the methods

WBC COUNTS ON PATIENT SAMPLES

Descriptive variables for WBC counts using different methods of estimation

Table 4, shows coefficient of variation (CV), mode, median, mean and standard deviation was calculated for total white blood cells (WBC) estimated values from samples of the 246 study participants. The values are for total WBC counts using the different methods of estimation; automated CD3200 and manual methods (Manual Native and Manual EDTA.

Table 4a: Mean and CV of total WBC counts by manual and automated methods (n=246)

Method	Mean+ SD	CV	Med	Mod
CD3200	9.3±6	67	7.4	6.3
CD1300	8.43±5	60	7	5.3
Manual Native	8.35±5	62	7	4.6
Manual EDTA	8.6±5.4	62	7.2	5.8
Neubauer Chamber	8.6±5.3	58	8.2	4.5

The mean WBC values ranged between $8.35\pm5 \times 10^9$ cell/l (NM) and $9.3\pm6 \times 10^9$ cells /l (CD3200). Overall, there was no significant difference between the means of total WBC counts estimated by CD3200, CD1300, NM, EM and NC (one way Analysis of Variance-ANOVA, F=1.214, ρ =0.3028). There was however significant difference among the SDs of the methods (Bartlett statistic = 11.321, ρ=0.0232) with the CD3200 showing the highest SD and CV.

WBC differentials

The same variables (mean, SD, CV, median and mode) were calculated for different WBC sub-populations (differential count) using automated CD3200 and manual methods (Manual Native and Manual EDTA). The values are represented in tables 4b-f for Neutrophils, lymphocytes, Monocytes, Eosinophils and basophils respectively.

Table 4b: Mean and CV of Neutrophil counts by manual and automated methods (n=246)

Method	Mean+SD	CV	Med	Mod
CD3200	61.1±19	31	60.5	59.6
Manual Native	61.5±17	27	60	56
Manual EDTA	61.5±17	27	60	52

There was no significant difference in CD3200, NM and EM mean percentage estimates for neutrophils ($F = 0.04192$; $\rho = 0.9590$). Bartlett statistic did not show a significant variation in the SDs of the percentage neutrophil estimates, $\rho = 0.1270$. Although the CVs were high, there was no significant variation in the modes and medians between the methods .

Table 4c: Mean and CV of lymphocyte counts by manual and automated methods (n=246)

Method	Mean+SD	CV	Med	Mod
CD3200	27.3±16	58	24.5	41.4
Manual Native	32±14	45	33	42
Manual EDTA	32.7±15	45	33	41

A significant variation was seen in CD3200, NM and EM mean percentage estimates for lymphocytes ($F = 9.4$, $\rho < 0.0001$) The variations were significant between mean estimates of CD3200 and each of NM and EM values but not among the manual methods. There was no significant difference in the SDs for lymphocytes ($\rho = 0.1136$) using the three methods. The CVs of lymphocyte estimates by NM and EM were similar but was high with CD3200.

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Table4d: Mean and CV of Monocyte counts by manual and automated methods (n=246)

Method	Mean+SD	CV	Med	Mod
CD3200	7.8±6	72	6.7	6.74
Manual Native	3.4±3	85	3	2
Manual EDTA	3.1±3	98	3	2

A significant variation was seen in CD3200, NM and EM mean percentage estimates for monocytes (F=94.619, ρ<0.0001). The variations were significant between mean estimates of CD3200 and each of NM and EM values but not among the manual methods. There was a significant difference in the SDs for monocytes (ρ<0.0001) There was a high variation in the CVs of monocyte estimates between the methods.

Table 4e: Mean and CV of Eosinophil counts by manual and automated methods (n=246)

Method	Mean+SD	CV	Med	Mod
CD3200	2.4±8	321	0.55	0
Manual Native	1.6±3	210	1	1
Manual EDTA	1.4±3	247	1	1

There was no significant difference in CD3200, NM and EM mean percentage estimates for eosinophils (F=2.520, ρ=0.0812). Bartlett statistic showed a significant variation in the SDs of the percentage for eosinophils ρ< 0.0001. Although the CVs were high, the modes and medians did not vary significantly between the methods.

Table 4f: Mean and CV of Basophil counts by manual and automated methods

Method	Mean+SD	CV	Med	Mod
CD3200	1.3±2	126	1.19	1.4
Manual Native	0	0	0	0
Manual EDTA	0	0	0	0

A significant variation was seen in CD3200, NM and EM mean percentage estimates for basophils (t=10.101, ρ<0.0001). The variations were significant between mean estimates of CD3200 and each of NM and EM values but not among the manual methods. No basophils were counted by the two manual methods.

Comparison of total WBC counts and differential counts using the reference methods: CD3200 (automated) and EM (manual)

Among the 246 samples analyzed, the mean total WBC counts were 8.6±5.4 and 9.3±6 for manual (EDTA) and automated (CD3200), there was no significant difference in the mean counts between the two methods (95% CI= -0.20 to 1.60, t = 1.53, ρ (two-tailed) = 0.13). Mean neutrophil absolute counts were 4.9±3.8 and 5.5±4.5 for EM and CD3200 respectively. The difference in the absolute neutrophil counts between the two methods was not statistically significant (95% CI= -0.14 to 1.34; t = 1.60, ρ (two-tailed) = 0.11; Figure 3).

Mean counts for the other cells were; lymphocytes were 2.5±1.3 and 2.2±1.3, monocyte 0.2±0.3 and 0.7±0.7, eosinophils 0.7±0.3 and 0.3±0.3 using EM and CD3200 methods respectively. The differences were statistically significant; lymphocytes (95% CI= 0.53 to -0.07; t = 2.56, ρ (two-tailed) = 0.01), monocytes (95% CI= 0.40 to 0.60; t = 10.30, ρ (two-tailed) <0.0001) and eosinophils (95% CI= 0.03 to 0.37; t = 2.35, ρ (two-tailed) = 0.02). The mean basophil count by CD3200 was 0.1±0.4 but there were no basophils counted by EM method (Figure 3).
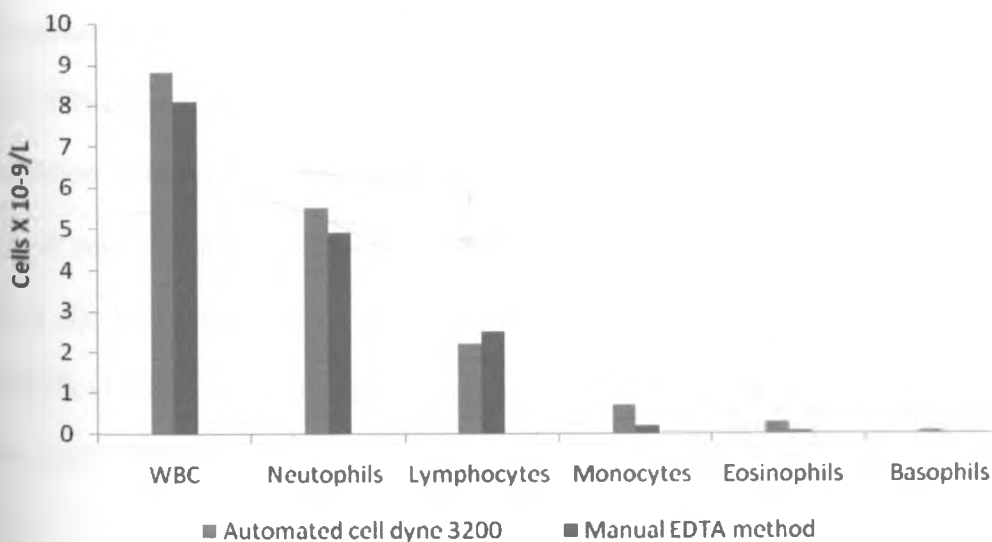

Figure 3: White cell counts by automated and   manual Method

Correlations of Total WBC counts of the reference methods: CD3200 and EM

The total WBC counts obtained with manual and automated counting methods on the EDTA-anticoagulated blood samples were nearly identical. There was a good correlation between the values of EM method and that of CD3200 hematology analyzer (r^2=0.952; linear regression line equation, y=0.845x+0.716; Figure 4).
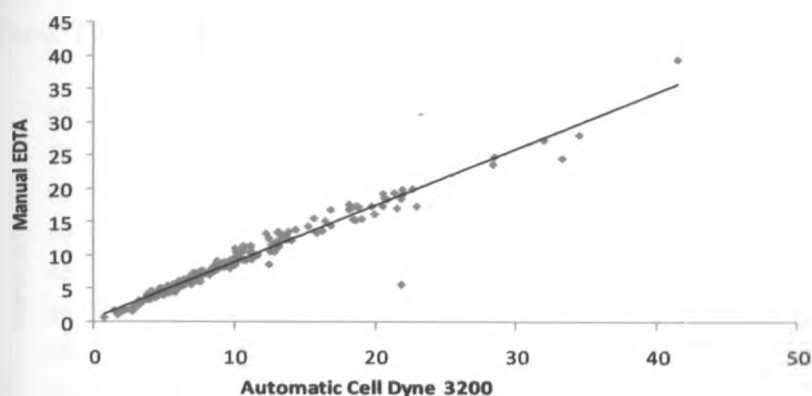

Figure 4: Correlation of total WBC counts (10^9cell/l) on the Cell dyne 3200 with manual microscopic method

Correlations of the differential WBC counts of the CD3200 and EM

In contrast, results of the differential cell counts obtained manually (EM) and with the automated (CD3200) method were not closely correlated as were total WBC counts. There was a good correlation between the percentage neutrophils determined manually and by the automated method was r^2=0.89 (y=841x+10.06; Figure 5) and lymphocyte, r^2=0.852 (y=0.874x+8.835; Figure 6). In contrast, there was a poor correlation for percentage monocytes (r^2= 0.238; y=0.267x+1.031; Figure 7) and eosinophils (r^2= 0.397; y=0.284x+0.707; Figure 8), respectively. There were no basophils counted by manual method (Figure 9).
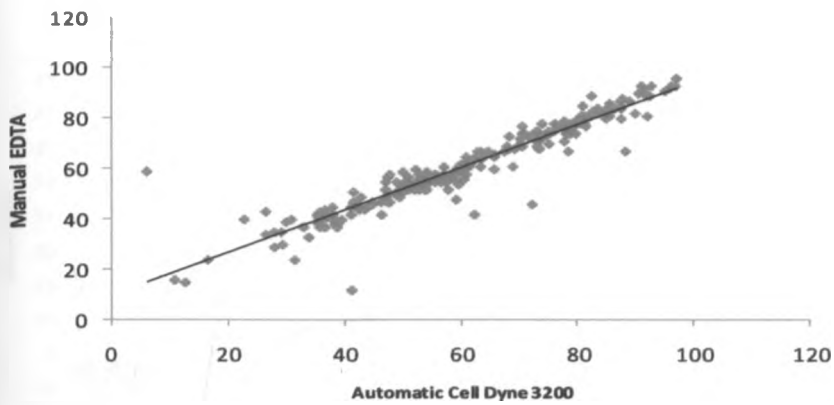
Figure 5: Correlation of neutrophils percentage on the Cell dyne 3200 with manual microscopic method. There was good correlation for neutrophils between the two methods, r2=0.89
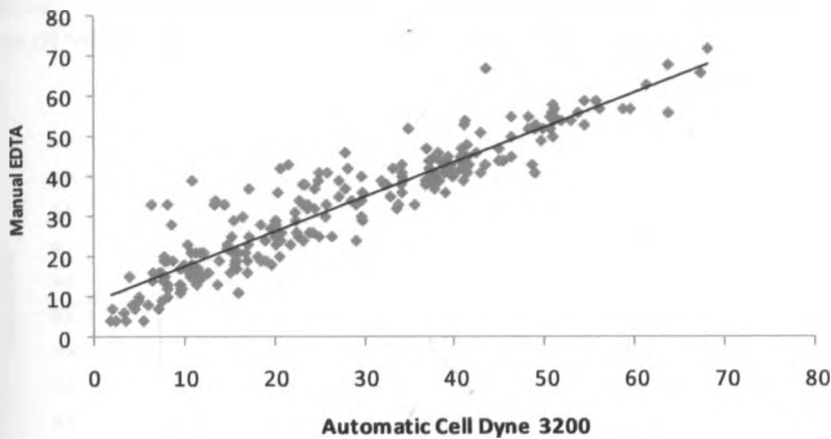

Figure 6: Correlation of lymphocytes percentage on the Cell dyne 3200 with manual microscopic method. There was good correlation for lymphocytes between the two methods, r2 = 0.852
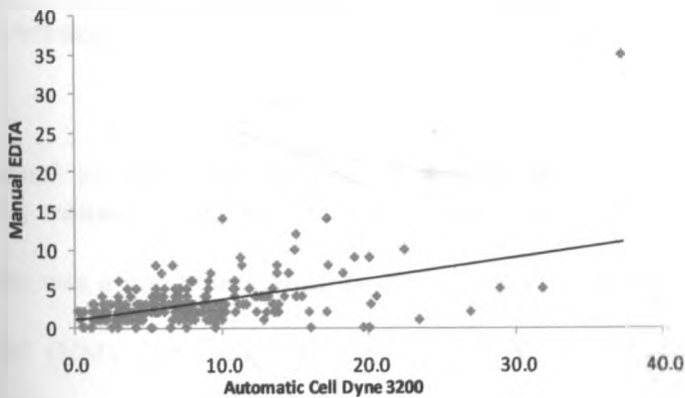

Figure 7: Correlation of monocytes percentage on the Cell dyne 3200 with manual microscopic method. There was poor correlation for   monocytes between the two methods, r2 = 0.238.
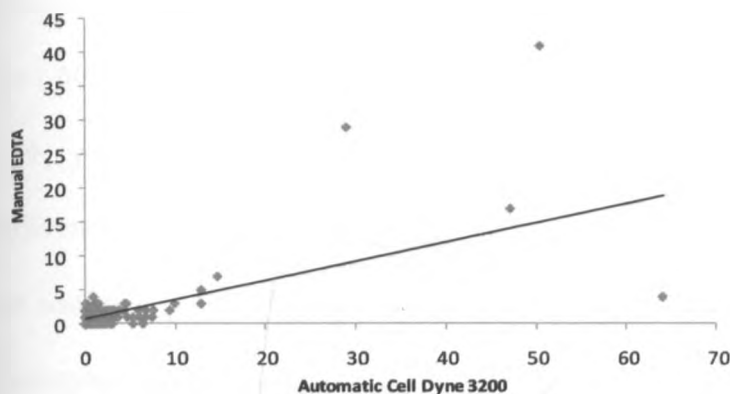
35

Figure 8: Correlation of eosinophils percentage on the Cell dyne 3200 with manual microscopic method. There was poor correlation for eosinophils between the two methods, r2 = 0.397 with some of values showing a wide scatter around the mean.
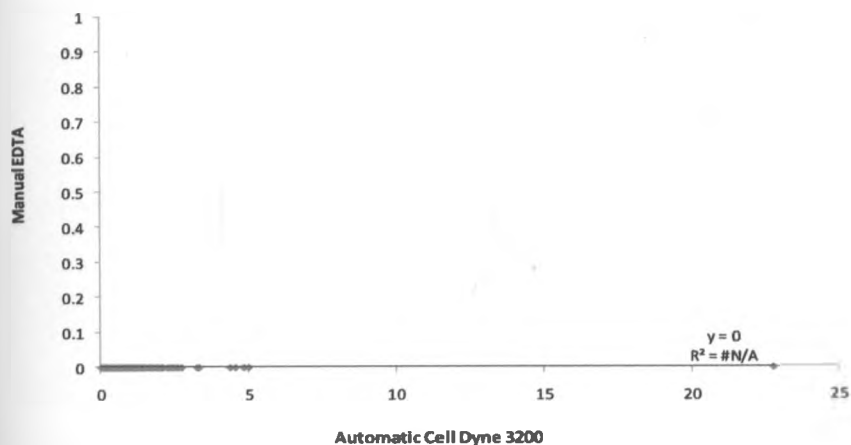
Figure 9: Correlation of basophil classification on the Cell dyne 3200 with manual microscopic method. No basophils were counted by the manual method hence correlation of the two methods was not done

Manual Method Comparison: Correlations between total WBC and differential counts using manual EDTA anti-coagulated and native (direct unanti-coagulated) blood

There was a good correlation in the total white cell count between the manual counts on native blood (NM) and EDTA anti-coagulated blood (EM). The correlation coefficient was 0.99 (y=1.051x-0.174) and there was no significant difference in the total WBC counts using manual

method in native blood and EDTA anti-coagulated blood (student's t-test, $\rho=0.59$; Figure 10A). Similarly, there was a good comparison in WBC differential counts by manual method using EDTA anti-coagulated and native blood. For neutrophils, the correlation coefficient was 0.99 ($y=1.007x-0.412$; Figure 10B), lymphocytes, 0.99 ($y=1.015x+0.153$; Figure 10C), monocytes, 0.91 ($y=0.921x-0.176$; Figure 10D) and eosinophils, 0.95 ($y=0.996x-168$; Figure 10E). The difference in the manual differential counts using EDTA anti-coagulated and native blood was not statistically significant, $\rho=0.99$, $\rho=0.61$, $\rho=0.1$ and $\rho=0.56$ for neutrophils, lymphocytes, monocytes and eosinophils respectively.
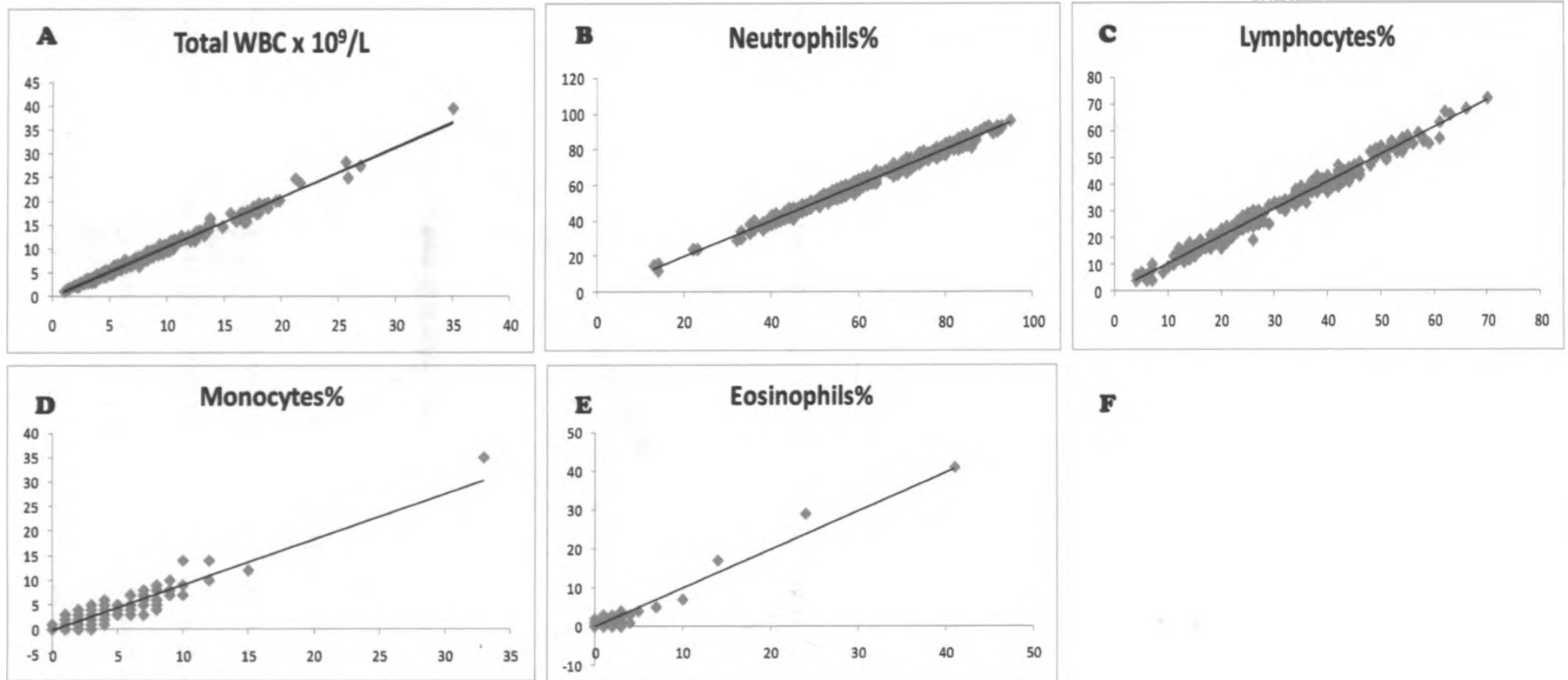
Figure 10: Correlation of cell counts by manual method using native blood (direct smear with fresh blood unanti-coagulated) and EDTA anti-coagulated blood. Y-axis represents the manual count of native blood while X-axis represents manual cell count of EDTA anti-coagulated blood. The Total WBC count is in 10^9cells/L while the specific (differential) cell counts are represented as a percentage of the total WBC. A-Total WBC; B-Neutrophils; C-Lymphocytes; D-Monocytes; E-Eosinophils

Comparison of total WBC counts between NC and the reference methods (CD3200 and EM)

When the total WBC counts were compared between NC estimate with CD3200 and EM, there was a good correlation, $r^2=0.951$ (y=0.838x+0.752). The correlation was even better with EM, $r^2=0.996$ (y=0.990x+0.049). There was no significant variation between the NC WBC estimation compared to CD3200 (Figure 20A) and EM (Figure 11B) ($\rho>0.05$).

Figure 11: Correlation of total WBC counts in 10^9cells/L by NC and other methods. A-NC *vs* CD3200; B-NC *vs* EM

COMPARISON OF OTHER BLOOD PARAMETERS, AGE, SEX AND DIAGNOSIS ON WBC COUNTS
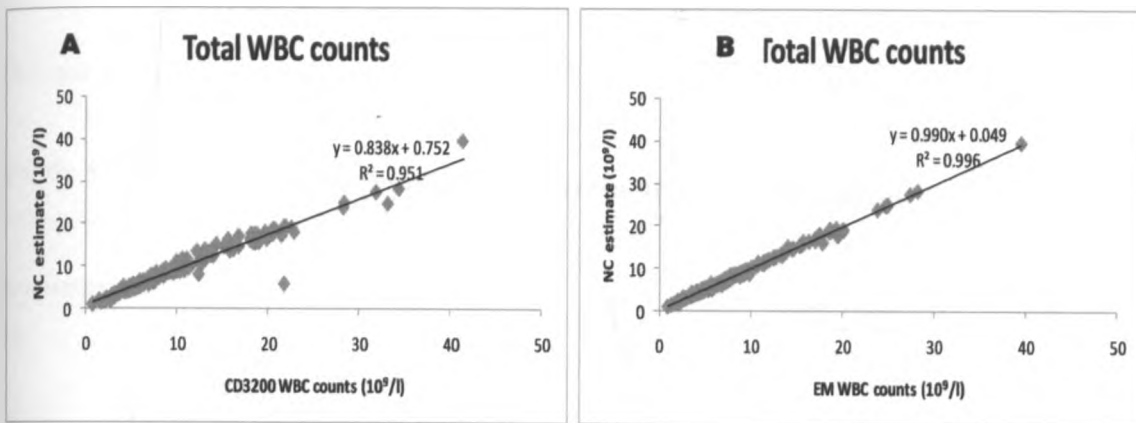
Table 5 below shows the effects of other haematological parameters on the Total WBC. Low RBc count was associated with a higher mean WBC count. ($p<0.05$). In contast, low platelet count was associated with lower mean WBC counts, while a high platelet count was associated with a higher mean WBC counts, this variation was significantly different ($p<0.05$). Increased ESR level was found to significantly affect the WBC count, with a mean increase in WBC in patients with higher ESR levels females ($p= 0.015$) but not in males ($p= 0.6127$). Low or high HB, PCV and MCV did not have an effect on WBC count. In addition, immature leucocytes and nucleated red blood cells did not appear to influence the WBC count.

Table 5: Effect of other haematological parameters on WBC count

Parameter	Level	N	WBC mean±SD	P
RBC	Low	59	11.5±8.1	0.035
	High	35	8.3±4.6	
HB	Low	110	10.3±7.5	0.9043
	High	0	10.0±8	
PCV	Low	86	11.0±8.1	0.6006
	High	10	9.6±6.7	
MCV	Low	14	8.2±2.7	0.116
	High	10	10.5±4.2	
Platelets	Low	21	6.8±7.2	0.0125
	High	62	11.3±6.9	
ESR	Normal female	20	6.4±2.9	0.015
	High female	105	10.5±7.3	
	Normal male	10	9.6±5.0	0.6127
	High male	111	8.7±5.4	
	Absent	76	9.1±6.1	0.6426
Immature WBC	Present	170	9.5±6.3	
NRBC	Absent	51	9.4±6.4	0.9189
	Present	195	9.3±6.2	

Total WBC and differential counts by sex

Screening by CD 3200, CD 1300, manual using native blood and manual using anti-coagulated blood methods had no significant differences in Total WBC count between males and females (p>0.05).Figures 12
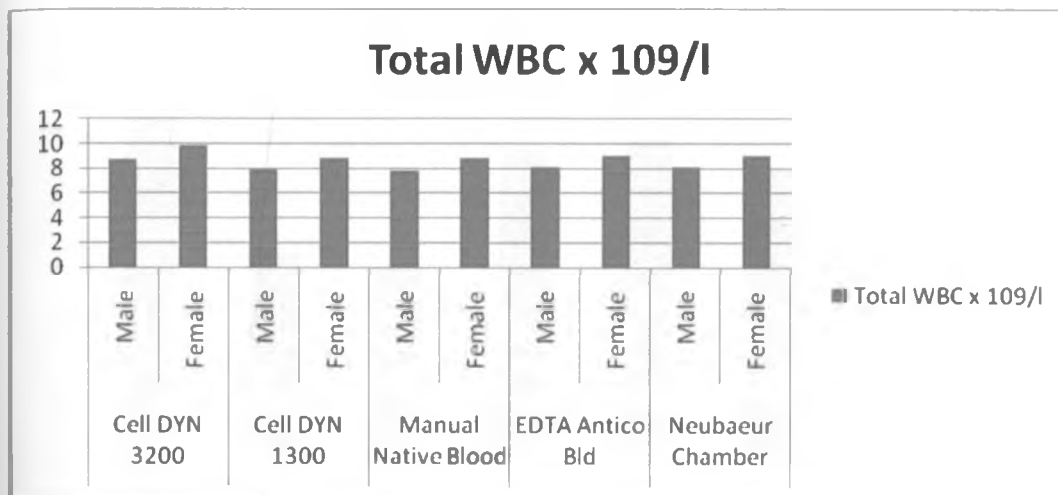
Figure 12: Comparison of WBC counts between males and females
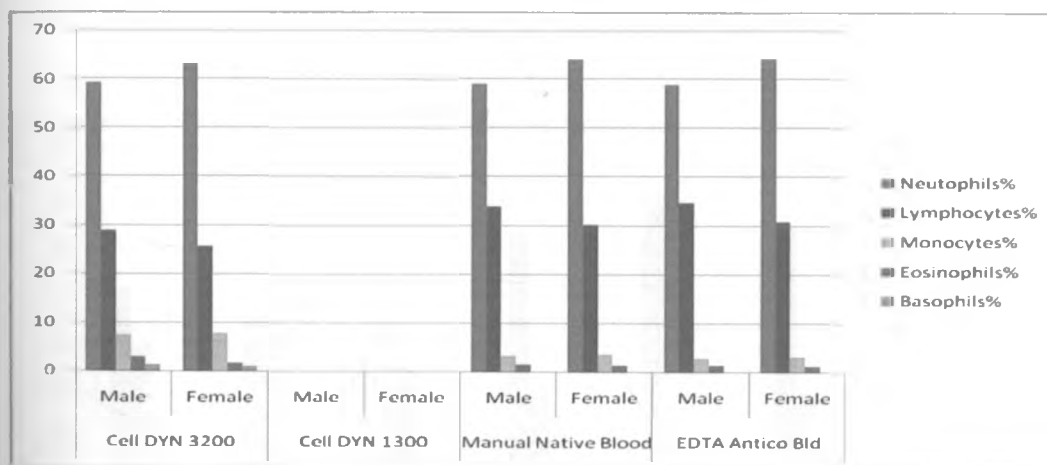
Figure 13: Comparison of differential WBC counts between males and females

Screening by CD 3200, CD 1300, manual using native blood and manual using anti-coagulated blood methods had no significant differences in WBC differential count between males and females. No counts were obtained with the Neubauer Chamber and CD1300 since they are used for TotalWBCcountsonly.

Total WBC and differential count by age

Total white blood cells counts showed a decreasing trend with increase in age up to the age of 10 years though not statistically significant. Children aged below 1 year had significantly higher WBC counts ($\rho<0.05$; figure 14).
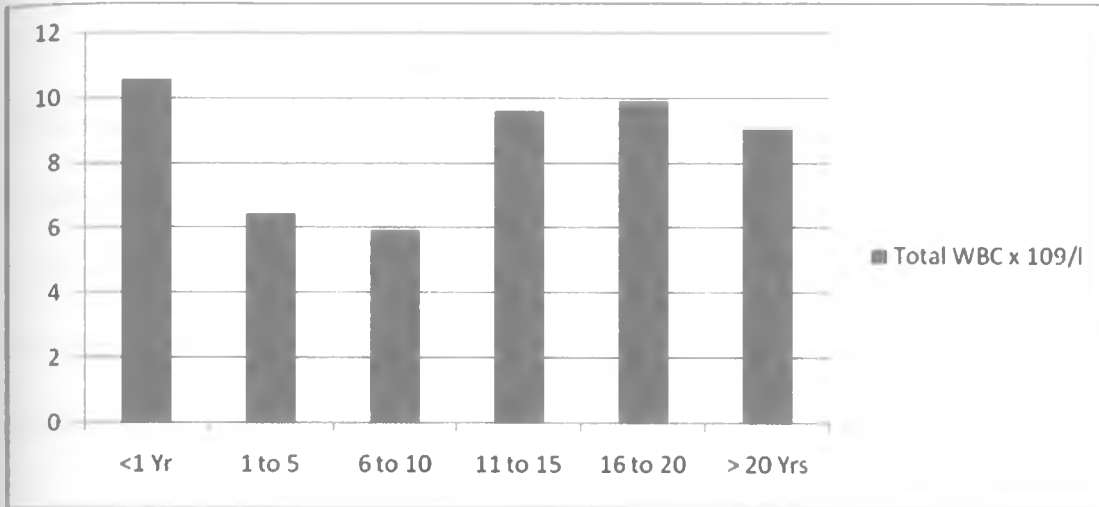
Figure 14: Total WBC counts by age
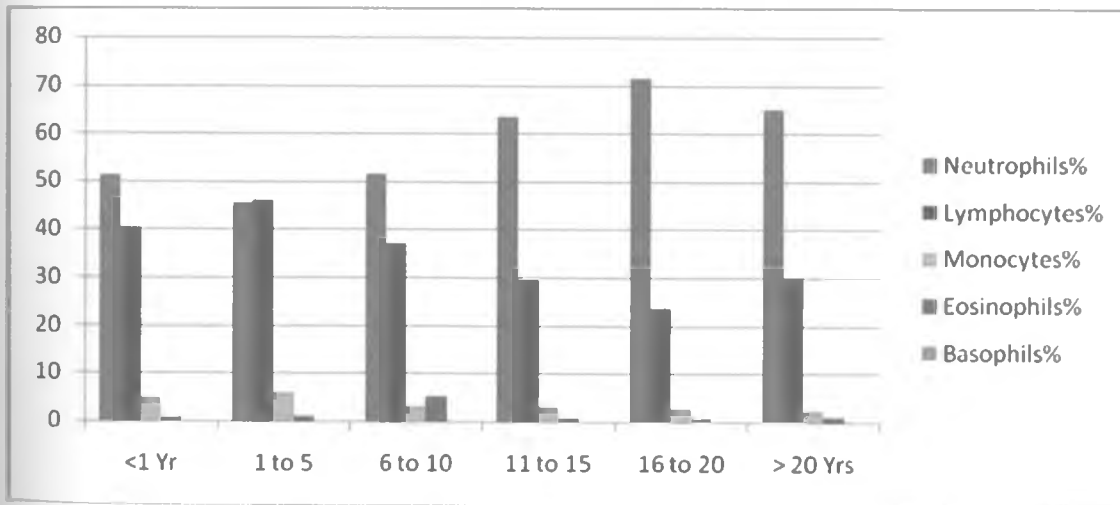
Figure 15: Differential WBC count by age

There was no trend for differential counts with age, but patients in the 16-20 years age group had the highest percentage neutrophil count (71.5%). In all age categories neutrophil counts were highest followed by lymphocyte counts (figure 15).

Comparing the different methods for total WBC counts by age

To determine the performance of CD 3200, CD 1300, NM and NC methods in detection of total white blood cells, different reference ranges were used for each specific age groups to test for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the test methods. The following WBC reference ranges were used for the different age groups; ages 0-14 days, 6.0 to 26.0×10^9/l, ages 14 days – 10 years, 6 to 14.5×10^9/l and over 10 years 3.5 to 11.0×10^9/l reference ranges were used. Values obtained from patients whose ages were not disclosed were not included in this analysis. All the methods had accuracy approaching 50% and sensitivities above 90% except in CD 3200 that had a sensitivity of 88% in ages 14 days to 10 years (table 6).

NB: The accuracy is estimated up to a maximum of 50. The methods with accuracy of 50 are estimated to have 100% efficiency.

Platelets distribution by age

The mean platelets in all age groups fell within the normal reference ranges. There was variation between age group although this did not show statistical significance ($\rho > 0.05$; figure 16).
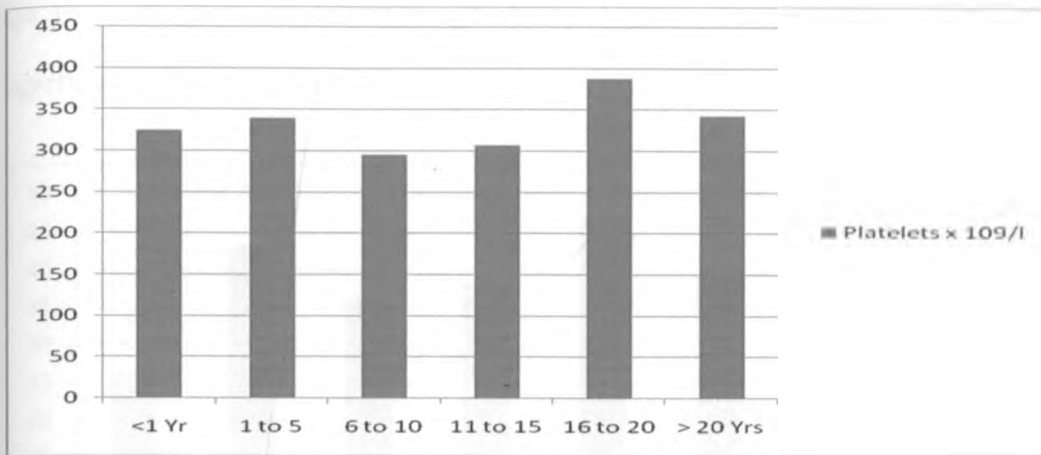
Figure 16: Distribution of the platelets (mean x 10^9/l) in different age groups

Erythrocytes sedimentation rates by sex

Erythrocytes sedimentation rates (ESR) were slightly higher among female patients than male patients though the difference was not statistically significant (p=0.571; figure 17).
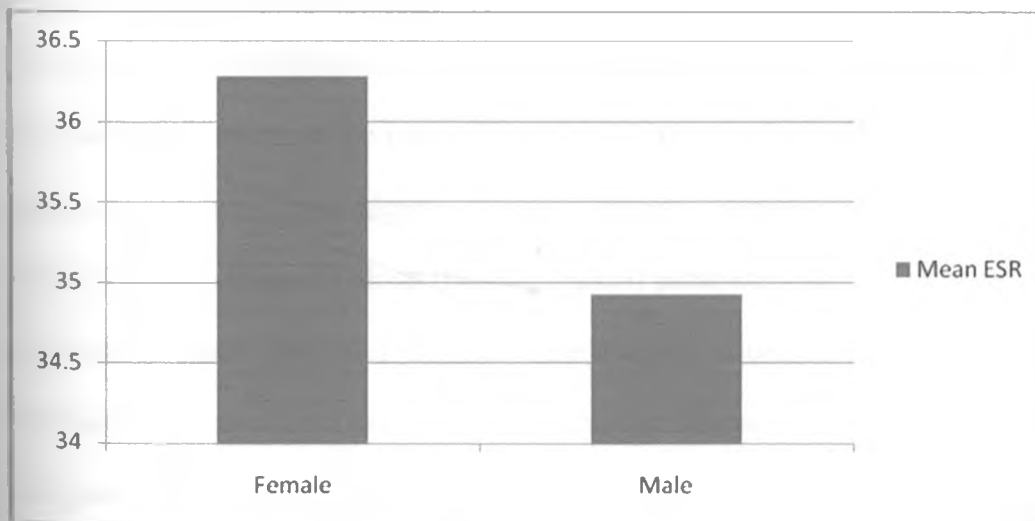
Figure 17: Comparison of Erythrocytes sedimentation rates (ESR) between males and females.

Erythrocytes sedimentation rates by age

Age was observed not to have an effect on the erythrocyte sedimentation rate. ESR was highest at age 16 to 20 years and lowest at ages below 1 year. The variation was though not statistically significant (p>0.05; figure 18)
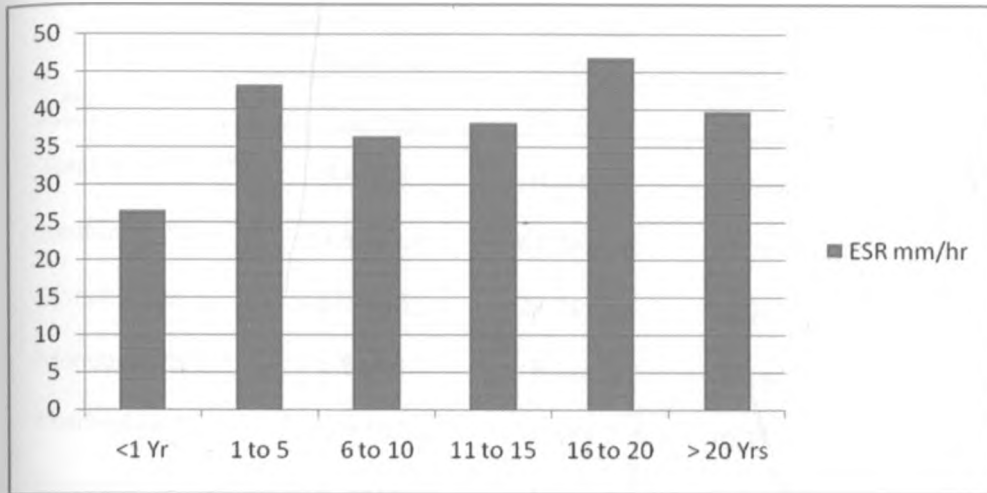
Figure 18: Variation of erythrocytes sedimentation rates with increase in age

WBC counts by diagnosis

Categorization of conditions affecting study participants

There were over 40 different disease states affecting the study cases and were grouped into four major categories based on the cause. These included hematological conditions, infectious diseases, neoplastic and non-communicable diseases (others). Hematological conditions were those of diseases causing change in blood (haematological) parameters and not necessarily associated with an infectious agent. Infectious diseases were diseases associated an infectious agent and can be communicable between persons. Non-communicable diseases were diseases not associated with an etiological agent some of which are inheritable. Diseases classified as neoplasia were tumors affecting the persons regardless of whether they were benign or malignant.

White cell values in relation to diagnosis

Majority of the participants had infectious diseases, 39.4% (97/246), followed by non-communicable diseases, 34.6 %(85/246). Hematological conditions were, 14.2% (35/246) while neoplastic diseases were 11.8% (29/246). (Table 7).

Table 7: Diseases groups affecting the study populations

	Haematological conditions	Infectious Diseases	Neoplasia	Non-communicable diseases	p
n (%)	35 (14.2)	97 (39.4)	29 (11.8)	85 (34.6)	p
Total WBC x 10^9/l	9.4±6.8	10.1±5.8	7.6±4.4	10.0±7.3	0.3
Neutophils %	61.4±18.4	61.2±20.6	51.5±17.9	64.8±17	0.01
Lymphocytes %	27.1±15	27.2±17.4	28.9±14.2	26.5±15	0.9
Monocytes %	6.8±4.8	8.5±6.3	8.1±6.8	6.4±3.9	0.06
Eosinophils %	3.6±10.8	1.1±1.7	10.3±19.7	1.3±2.1	<0.001
Basophils %	1.1±0.6	1.3±1.1	1.2±0.5	1.0±0.6	0.1

Generally, there was no significant difference in mean WBC count in between the four groups of disease (ρ=0.3) but there was a significant variation in the mean percentage estimates for neutrophils (ρ=0.01) and eosinophils (ρ<0.001) between the groups. Variation in the mean percentages of other WBC populations was not significantly different among the groups

Proportions of different hematological conditions

Table 8 below shows the different hematological conditions identified in the study participant. Of the 35 participants with hematological conditions, majority (42.9%) had ISS/Anaemia. Anaemia alone was also common (28.6%) among these patients. Other conditions identified are as shown in table 8.

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Table 8: Haematological conditions identified among the participants

Prevalence of haematological Conditions	Frequency	Percentage
AML	3	8.6
ALL	1	2.9
CML	2	5.7
CLL	1	2.9
Anaemia	10	28.6
ISS/Anaemia	15	42.9
Sickle cell disease	1	2.9
Lymphomas	2	5.7
Total	35	100.0

Association of total WBCs and differential WBC counts with infectious diseases

Infectious diseases were categorized based on whether the etiology was bacterial, fungal, viral or parasitic. Bacterial infections were more common than other infections in this group accounting to 85.6% of the total patients with infectious diseases. The other infections were less common and included; parasitic (4.1%), viral (3.1%) and fungal (2.1%). Five (5.2%) of the 97 participant with infectious diseases had co-infection of parasitic and bacterial agent; this involved malaria associated with different bacterial agents (table 9).

Table 9: Relation between total and differential WBC with infectious diseases

			Infections			
	Bacterial	Fungal	Parasitic	Viral	Parasitic/Bacterial	
n (%)	83 (85.6)	2 (2.1)	4 (4.1)	3 (3.1)	5 (5.2)	p
Total WBC x 10^9/l	10.5±5.7	6.4±3.2	8.3±8.2	5.3±1.7	6.5±2.1	0.17
Neutrophils	62.6±20.8	60.2±11.9	62.7±15.8	49.1±6.9	39.5±7.9	0.1
Lymphocytes	25.7±17.3	20.7±23.3	28.5±17.6	37.6±11.2	47.5±5.9	0.06
Monocytes	8.6±6.5	5.0±6.3	7.1±2.8	9.2±7.3	10.4±3.1	0.86
Eosinophils	1.1±1.7	1.0±1.5	0.4±0.4	2.1±1.4	1.1±0.9	0.76
Basophils	1.3±1.1	0.8±1.0	1.4±1.0	2.4±0.1	1.6±0.5	0.42

Mean total WBC counts were higher in bacterial infections than they were in other conditions, the variation of total WBC between the infectious diseases was not statistically significant ($p=0.17$). In addition, neutrophils were high in fungal infections; no other cell population was increased in relation to a particular disease. There was no significant association of any disease to a specific WBC type ($p>0.05$)

Immature white blood cells

Immature white blood cells (blasts and band forms) were evaluated by CD3200, NM and EM. Using CD3200 immature cells were seen in 76 (30.9%) compared to 101 (41.1%) in NM and EM, 96 (39%). Significantly more immature cell were seen using manual method on native than other methods ($x^2=6.104$, $p=0.0473$), CD3200 had the lowest count for immature cells (figure 19).

addition, NM had a higher accuracy compared to CD3200 in detection of immature white cells and NRBC as shown in table 10.

NB: The accuracy is estimated up to a maximum of 50. The methods with accuracy of 50 are estimated to have 100% efficiency.

Table 10: Sensitivity and specificity of CD 3200 and Manual using native blood methods

Cell types	Method	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
BLASTS	CD 3200	83	53	73	67	35
	Manual Native	85	82	88	78	42
NRBC	CD 3200	80	33	96	5	39
	Manual Native	96	67	98	37	47
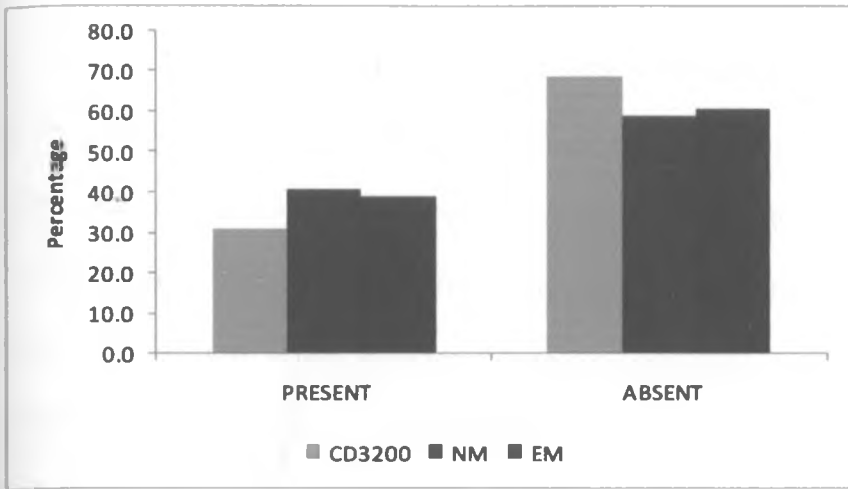
Figure 19: Presence or absence of immature cells using CD3200, NM and EM methods.

Nucleated red blood cells

Nucleated red blood cells (NRBC) were not commonly observed. Fifty one (20.7%), 16 (6.5%) and

9 (3.7%) had NRBC using CD3200, NM and EM, respectively. Significantly more NRBC were

counted using automated than the manual methods (χ^2=44.563, ρ<0.0001; figure 20).
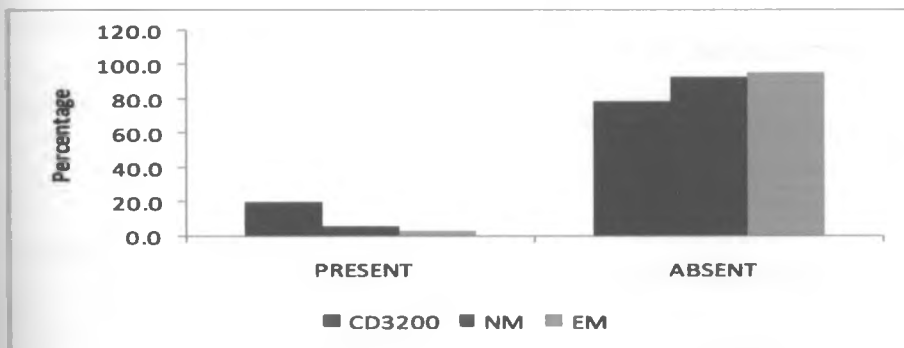
Figure 20: Presence or absence of NRBC using CD3200, NM and EM methods.

Sensitivity and specificity for CD3200 and NM methods in screening for immature white cells and NRBC

Tests of performance of the two methods against EM as gold standard showed that NM had a

higher sensitivity (85%) and specificity (82 %) for immature white cells detection than CD3200.

Similarly NM had a higher sensitivity (96%) and specificity (67 %) for NRBC detection. In

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7 DISCUSSION

Instruments capable of performing automated counts of blood cells have been available since the 1980s[56]. Automated hematology analyzers with ability to do a full blood count (FBC) and differential estimation of WBC populations have replaced the traditional manual or individual assay methods for hematologic parameters and the eye count WBC leukocyte differential as the initial screening and detection system for hematologic abnormalities in modern hospitals and clinics. The traditional review of all automated hematology instrument results by preparation, staining, and microscopic examination of a blood film has disappeared in most institutions. This is because of the high accuracy of the automated analyzers compared to manual eye count method [47]. There are however some cellular abnormalities that may fail to be identified by the automated methods hence calling for human judgment. In the current study, automated methods were compared to manual methods and the results indicated none was superior to the other. This correlates with a different study by Barrett [57] where pleural fluid WBC counts obtained with manual and automated counting methods on EDTA-preserved fluid samples were nearly identical. Manual counting is a costly, time-consuming, labor-intensive laboratory procedure, which is dependent on the availability of skilled laboratory personnel. Its correlation with automated methods is thus important since automated methods can replace the manual methods without compromising the quality of results.

The coefficients of variation (CVs) of the control samples for TWBC, neutrophils and lymphocytes correlated with those of a study done in China assessing the CVs among machines by different manufacturers (Sysmex, Beckman Coulter and Abbott) found CVs of 3.2%, 3.8%, 3.6%, 9.3% and 10.8% for measurement of RBC, Hb, PCV, WBC and platelet count, respectively [45]. The CVs of the WBC for these methods were similar to those found in this study.

In contrast, both methods (CD3200 and EM) had a high CV (>20%) for monocytes, eosinophils and basophils and therefore are less accurate in estimation of these parameters. This is further

confirmed by the fact that the statistical difference between manual and automated methods was observed in the mean control values of monocytes, lymphocytes, monocytes and lymphocytes but not in total WBC count, and neutrophils. These high CVs would be associated with small number of cells (100 WBCs/ PBF) evaluated by the manual methods.

The CV values for patients were high (>25%) for all the parameters tested using different methods. This difference may be related to the variations in the cell count of the patients who had different disease conditions. In this study, NRBC counted by both methods did not seem to interfere with the WBC counts. The mean white cell count in samples with or without NRBC were similar (p>0.05) even after correcting for the total WBC counts where it was found necessary. Some authors [13] have reported that immature WBCs and nucleated RBCs interfere with the automated WBC counts due to presence of nuclei and leave a WBC like particle when lysed [14]. The small number of these cells may have contributed to the lack of interference with WBC count. Hb, PCV and MCV did not interfere with the WBC counts as well; this could be because none of the parameters can be counted as WBC. However, high ESR was associated with high WBC counts in females than in males. The reason for this is observation is not clear. Low platelet counts were associated with reduced mean WBC; the reason for this observation is unclear. Low RBC was associated a higher mean WBC while high RBC count was associated with higher mean WBC count. The possible reason for this is that some RBC were partially lysed and counted as WBC or conditions causing reduced red cells may be associated with increased white cells and vice versa. With no observable trend in blood cell counts with age increase or sex it may imply that other factors like disease conditions or disease durations may have a greater contribution to the counts. Among four categories of diseases affecting our study cases, there was no significant difference in mean WBC count in between the four groups of disease (ρ=0.3) and therefore there was no particular group that could be associated with as significant rise in WBC compared to others.

There was a significantly lower level of neutrophils and increased eosinophils in patients with neoplasia. There was no infection that was associated with increased level of particular cell type among the patients; this contradicts the theory that certain infections are associated with increase in the specific cell types. This contradiction may be explained by the fact that some of the participants had dual or multiple disease conditions.

Where the manual methods using EDTA anti-coagulated blood (EM) and native unanti-coagulated blood (NM) were used for estimation of the counts, the TWBC and WBC sub-populations highly correlated ($r^2 > 0.91$) and inter- manual variations were not significant in the mean estimates of these cell populations ($p > 0.05$). This indicates that when preparing blood films for manual estimate of total WBC and differential counts adding anticoagulant is not necessary and blood films prepared directly prior to anticoagulant addition produce similar results as EDTA anti-coagulated blood.

In the comparison of WBC values using the EM and CD3200, the results for total WBC were highly correlated ($r^2 = 0.952,$) with small dispersion. Similarly, neutrophil and lymphocyte counts between the two reference methods showed little dispersion and a high correlation ($r^2 > 0.8$). A different study in Germany evaluated the performance of CELL-DYN 3200 comparing it with microscopy results (400 cells). Linear regression coefficients were 0.96, 0.95, 0.68, 0.95, and 0.57 for neutrophil, lymphocyte, monocyte, eosinophil, and basophil percentages, respectively [38]. The was an agreement between the finding of the German study and the present study in differential neutrophil and lymphocyte count. This means that the manual method (EM) would be used with confidence to give similar results as the automated CD3200 machine for estimation of total WBC, neutrophil and lymphocytes.

In contradiction, there was poor correlation between manual and CD3200 estimation of monocytes, eosinophils and basophils ($r^2 < 0.5$) in this study. Manual method did not identify basophils in the blood film. The difference may result from the limited number of cells counted (100 cells) in

this study as opposed to the German study where they counted 400 cells. Results similar to those of the current study were obtained in a study by Post et al.[58] who found discrepancies between the automated and manual methods for monocytes, eosinophils, and basophils and found lower percentages monocytes count by manual method. This lack of agreement between methods would be attributed to monocytes being not equally distributed over the edge of the film and are easily missed during counting as well as small number of total cells counted. In addition, the discrepancies may also be related to difficulties in identification the blood cell types with manual method, especially for basophils due to their paucity in the specimens.

Neubauer chamber method of crude WBC estimation showed a good correlation with other methods indicating its suitability for use in estimation of WBC. Despotis et al.[59] showed that the NC was accurate in estimation of WBC, RBC and platelets when compared with an automated method. These results were in agreement with findings of this study showing the appropriateness of NC in estimation of WBC especially in poor resource settings as well as an important tool for use in point of care testing in critically ill patients.

When manual (EM) was used as a gold standard for assessing the performance of other methods in assessing low and high WBC counts and flagging of atypical cells (immature WBC and NRBC). All the methods performed well with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) above 85% and accuracy above 40%. For WBC count, NC showed the highest specificity, sensitivity, positive predictive value, negative predictive value and accuracy. This went against the perception that NC is a crude method of estimation of WBC. Results of this study support the suitability of the NC in estimation of total WBC count. CD3200 on the other hand performed least among the methods used and this could be as a result of using manual method as the gold standard for comparison.

In assessment of performance of flagging for immature granulocytes and NRBC; manual method using native blood (NM) performed better than automated flagging for immature cells and

NRBC. The performance was poorer in detection of NRBC than immature WBC. This could be explained by lack of differentiating power in differentiation of these cells by machine as opposed to the eye judgment. According to this study, the manual methods would therefore remain preferable to automated methods for counting the immature WBC and NRBC.

The hypothesis of this study was that, "there are no differences in white blood cell values as estimated by automated and manual methods". Findings of this study did not find statistically significant variation in WBC counts between manual and automated methods and therefore **"fail to reject the Null hypothesis"**

STUDY LIMITATIONS

- Delays were occasioned by the frequent lack of reagents for the automated counter,CD 3200

8 CONCLUSIONS

Based on the findings of this study, the following inferences can be made;

1. Manual methods produced comparable results with automated method (CD3200) for estimation of total WBC, lymphocytes and neutrophils.

2. Manual estimation of differential cell counts other than neutrophils and lymphocytes showed less accuracy due to the small number of cells counted.

3. Manual estimation using anti-coagulated blood and native blood was comparable, obviating the need to anti-coagulate blood for making blood film especially when blood is only required for this purpose, a finger prick can be used.

4. Compared to manual methods, CD3200 had less accuracy in flagging immature white cells and nucleated red cells.

9 RECOMMENDATIONS

- The study recommends use of the automated method (CD3200) for total and differential WBC counts. However, the study also recommends use of manual methods where facilities for automation are not available or in situations where the automated machine (CD1300) is incapable of doing differential counts.

- Manual estimation can be used to compliment automated machine or used in place of automated machine in case of machine downtime with similar accuracy expected.

- In manual estimations of WBC values, native blood film obtained from a finger prick is as good enough as compared to EDTA anti-coagulated blood film. Again, this is recommended in resource poor settings.

- There is need to count more WBC while performing the manual counts on peripheral films in order to reduce the error of distribution especially for monocytes, eosinophils and basophils. It is recommended that the routine 100 WBC count be avoided in favour of 200 cell count.

- It is recommended that manufacturer of the automated systems should highlight/give details in their methodology inserts on the suspects/factors affecting WBC counts especially when other cell parameters eg NRBC, large platelets have been shown to interfere with overall WBCevaluation.

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APPENDIX I: PROFORMA QUESTIONNAIRE

AUTOMATED AND MANUAL WHITE BLOOD CELL COUNTS IN KNH.

Date of study ☐☐☐

Date of report ☐☐☐

Study Number ☐☐☐

Lab. Number ☐☐☐☐

Inpatient (IP) Number ☐☐☐☐☐☐

Patient information;

Name ……………………………………………………

Address …………………………………………………

Occupation ………………………………………………

Sex ……………………………….

Age …………………………….

Ward ……………………………………………..

Requesting Doctor ………………………………………

Clinical summary ………………………………………………

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…………………………………………………………………

Diagnosis…………………………………………………………

Laboratory findings (Manual)

	PBF Native blood	PBF Anti-coagulated	Neubauer chamber counts
Total WBC Counts (x 10^9/L)	☐	☐	☐
Corrected WBC Counts (x 10^9/L)	☐	☐	

Differentials :

.Neutrophils (%)	☐	☐
.Lymphocytes (%)	☐	☐
.Eosinophils (%)	☐	☐
.Monocytes (%)	☐	☐
.Basophils (%)	☐	☐

Other parameters:

Immature WBC (per 100WBC)	☐	☐
NRBC(per 100WBC)	☐	☐
Platelet counts (x 10^9/L)	☐	☐

Laboratory findings (Automated)

	CELL DYN 1300	CELL DYN 3200
Total WBC counts (x 10^9/L)		

Differential counts:

Neutrophils (%)	
Lymphocytes (%)	
Eosinophils (%)	
Monocytes (%)	
Basophils (%)	
Immature WBC (%)	
NRBC (%)	
Corrected WBC Counts (x 10^9/L)	

Platelets (× 10⁹/L)

Red cell parameters;

a) Hb

b) RBC

c) PCV

d) MCV

Other parameters;

a) Erythrocyte sedimentation rate (mm/hr)

APPENDIX II: CONSENT FORM

COMPARATIVE ASSESSMENT OF AUTOMATED AND MANUAL WHITE BLOOD CELL COUNTS AT KENYATTA NATIONAL HOSPITAL HAEMATOLOGY LABORATORY

My name is Dr Muturi, C.K. and I am carrying out the above study to compare the white blood cell values obtained by automated and manual methods. There is no risk that shall be involved in this study. The study shall benefit you and shall be done free of charge, and the results were communicated to your doctor who shall then give you the feedback. It will help us in deciding which method is most appropriate in our setting. I request your permission to use your blood in performing the study.

The participation in this study is voluntary and one is allowed to withdraw at any time and this shall not interfere with management of your illness by your doctor.

I .... do consent to participate in this study being carried out by Dr Muturi, C.K. I have asked questions and everything has been explained well by him.

Date ....

Signature of patient/guardian ....

If you have any concerns about this study you can contact the chairperson of the Ethical and Research Committee at KNH on Tel.

You may also contact my study supervisors;

Prof W.O. Mwanda Tel 2721815
Haematology Unit, Department of Human Pathology
University of Nairobi

Dr F.K. Abdallah Tel 2721815
Haematology Unit, Department of Human Pathology
University of Nairobi

My contact as the principal investigator is 0733834101

APPENDIX III A: Specimen processing for manual white blood cell count (Neubauer chamber)

- .Make a 1 in 20 dilution of blood by adding 0.1ml of well mixed blood to 1.9ml of lysing fluid (2% (20ml/L) acetic acid coloured pale violet with Gentian Violet) in a 75 x 10mm glass/plastic tube.
- Seal tube with a lid / bung, mix the diluted blood in a mechanical mixer or by hand for at least 2 minutes.
- Fill a clean dry Neubauer counting chamber using Pasteur pipette/ stout glass capillary, with its cover-glass already in position, without delay, while ensuring that no fluid flows into the surrounding moat.
- Leave the chamber undisturbed on a bench for at least 2 min for the cells to settle
- The preparation should be viewed with the 4mm objective and x6 eye-pieces or the 16mm objective and x 10 eye- pieces
- Count the cells in the entire ruled area(i.e 9 x0.1ul areas in an improved Neubauer counting chamber)
- Calculation:

White blood cell count per litre (WBC/L) =

No. of cells counted   x Dilution x 10^6

Volume counted (ul)

Thus if N cells are counted in 0.1ul, then the WBC/L is :

$\underline{S}$ x 20 x 10^6 = N x 200 x 10^6

0.1

e.g. if 115 cells are counted, the WBC is 115 x 200 x 10^6/ L = 23 x

10^9/ L

APPENDIX III B: Specimen processing

1) Specimen processing methodology for manual counts (PBF)

- Blood films should be made on clean glass.
- Blood used is either fresh with no anticoagulant added or from EDTA-anticoagulated blood.
- Place a small drop of blood in the centerline of a about 1 cm from one end.
- Without delay, place a spreader in front of the drop at an angle of about 30º to the slide and move it back to make contact with the drop.
- With steady movement of the hand, spread the drop of blood along the slide. The spreader must not be lifted off until the last trace of blood has been spread out.
- Label the film immediately after spreading, on the frosted edge of the slide.
- Let the films dry in air.
- The films are then fixed by the use of methyl alcohol or ethyl alcohol(absolute alcohol) for 5-10 minutes.
- Transfer the film into a jar containing May-Grunwald stain freshly diluted with an equal amount of buffered water, and allow it to stain for 15 minutes.
- Without washing, transfer the slide into a jar containing giemsa stain freshly diluted with 9 volumes of buffered water, pH 6.8 and allow to stain for 10-15 minutes.
- Transfer the slide onto a jar containing buffered water at pH 6.8 and wash in three to four changes of water and then allow to stand in water(for 2-5 minutes) for differentiation to take place.
- Once differentiation is complete, stand the slides upright to dry.
- Examine the film at high power and count at least 100 white cells. This gives the percentage of cells that are of each type. By multiplying the percentage with the total number of white blood cells, the absolute number of each type of white cell can be obtained.

- **Correcting the count for nucleated red blood cells (NRBC)**
  When the NRBC are present, they were included in the total WBC, which is really a total nucleated cell count (TNCC). They should also be included in the differential count as a percentage of the TNCC and reported in absolute numbers (x 10^9/L) in the same way as the different types of leucocytes. If they are present in significant numbers, the TNCC should be corrected to obtain the true total WBC. Thus for example if the total WBC is 8.0 x 10^9/L and the percentage of NRBCs on the differential count is 25%, then corrected WBC = 8 – (8 x 25) = 6 x 10^9/L

  100

  NB;Care should be taken to differentiate small lymphocytes from NRBC

2) Formula for estimation of Total White Cell counts in Peripheral Blood Film (PBF)

At x 40 and x 100 magnification count the number of white cells in 5 fields and get the average and then multiply by 1.5 and 3.5 respectively. This will give an estimate number of cells (n) x 10^9/l .

3) Procedure for Erythrocyte Sedimentation Rate (ESR)

- The test is performed on venous blood diluted accurately in the proportion of 1 volume of citrate to 4 volumes of blood. 109mmol/l or 32g/l trisodium citrate is used as the anti-coagulant diluent solution. The test can be carried out equally well with blood anti-coagulated with EDTA within 24 hr if the specimen is kept at 4o C, provided that 1 volume of 109mmol/l (32g/l) trisodium citrate is added to 4 volumes of blood immediately before the test is performed
- Mix the blood sample thoroughly and then draw it up into the Westergren tube to the 200 mm mark by means of a teat or a mechanical device
- Place the tube exactly vertical and leave undisturbed for exactly 60 min, free from vibrations and draughts, and not exposed to direct sunlight.
- Then read to the nearest 1 mm the height of the clear plasma above the upper limit of the column of sedimenting cells.
- The result is expressed as ESR = X mm in 1 hr.

NB; A poor delineation of the upper layer of red cells may sometimes occur, especially when

there is a high reticulocyte count.

# APPENDIX IV A: Principle of CELL DYN 3200 counter

- The CELL-DYN 3200 uses flow cytometric techniques to analyze the RBC, WBC and PLT populations. Flow cytometry is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light.

- A sensor or sensors measure, by the loss or scattering of light, the physical or chemical characteristics of the cells or particles.

- Flow cytometry enables the rapid screening of large numbers of cells and provides quantitative cell analysis at the single-cell level. The basic components of a flow cytometer include:

   o A sample collector and transporter

   o A flow system to focus the sample flow stream

   o A light source and focusing optics

   o Light collectors, signal detectors, and polarizers

   o Data collection and storage

   o Data display and analysis

- In a flow cytometer, the cell suspension is transferred from the mixing chamber through a sample tube into a special flow chamber with a small opening at the tip. The suspension is then injected into a stream of fast-moving, cell-free liquid (sheath fluid). Since the two liquids travel at different rates of speed, they do not intermingle.

- The special geometry of the flow cell and the flow rate of the sheath fluid forces the cells into single file. This process is known as hydrodynamic focusing.

- As the cells enter the view volume (specific viewing area), they intersect with the laser beam.

- The different types of cells scatter the laser light at different angles, yielding information about cell size, internal structure, granularity and surface morphology.

- The optical signals the cells generate are detected and converted to electrical impulses which are then stored and analyzed by the computer.

- Flow cytometers generally measure two angles of scatter. Forward angle light scatter is a measure of cell size. Side angle (orthogonal) light scatter is a measure of cell surface and internal structure but is primarily a measurement of internal granularity. Combining the information from the two scatter measurements provides more accurate discrimination between cell populations than either single measurement.

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1 Sample Feed Nozzle
2 Sheath Stream
3 Sample Stream
4 Focused Laser Beam
5 Various Angles of
  Scattered Light
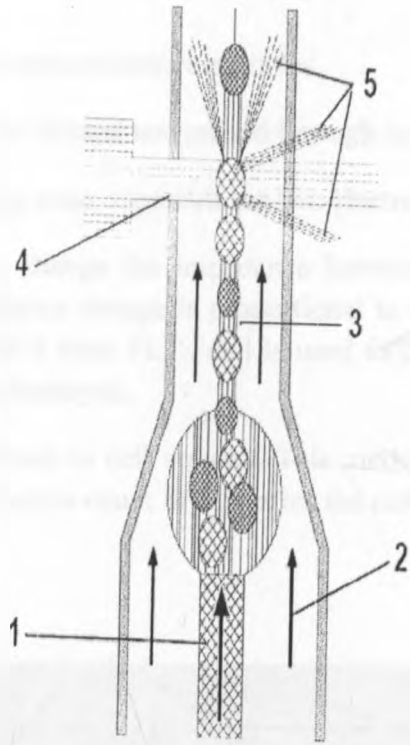
APPENDIX IV B: Principle of CELL DYN 1300 (Impedance counting)

- Impedance counting treats cells as nonconductive particles.

- They are suspended in a fluid called diluent and passed through an aperture.

- The aperture also called the sensing zone, separates the two electrodes.

- Cells passing through the aperture change the impedance between the electrodes and this impedance is measured. The impedance change is proportional to cell volume. This method constitutes one way to separate RBCs from PLTs and is used to count WBCs after the cell membranes of the RBCs have been destroyed.

- The impedance change is proportional to cell volume. This method constitutes one way to separate RBCs from PLTs and is used to count WBCs after the cell membranes of the RBCs have been destroyed.

Counting and sizing blood cells – Impedance method
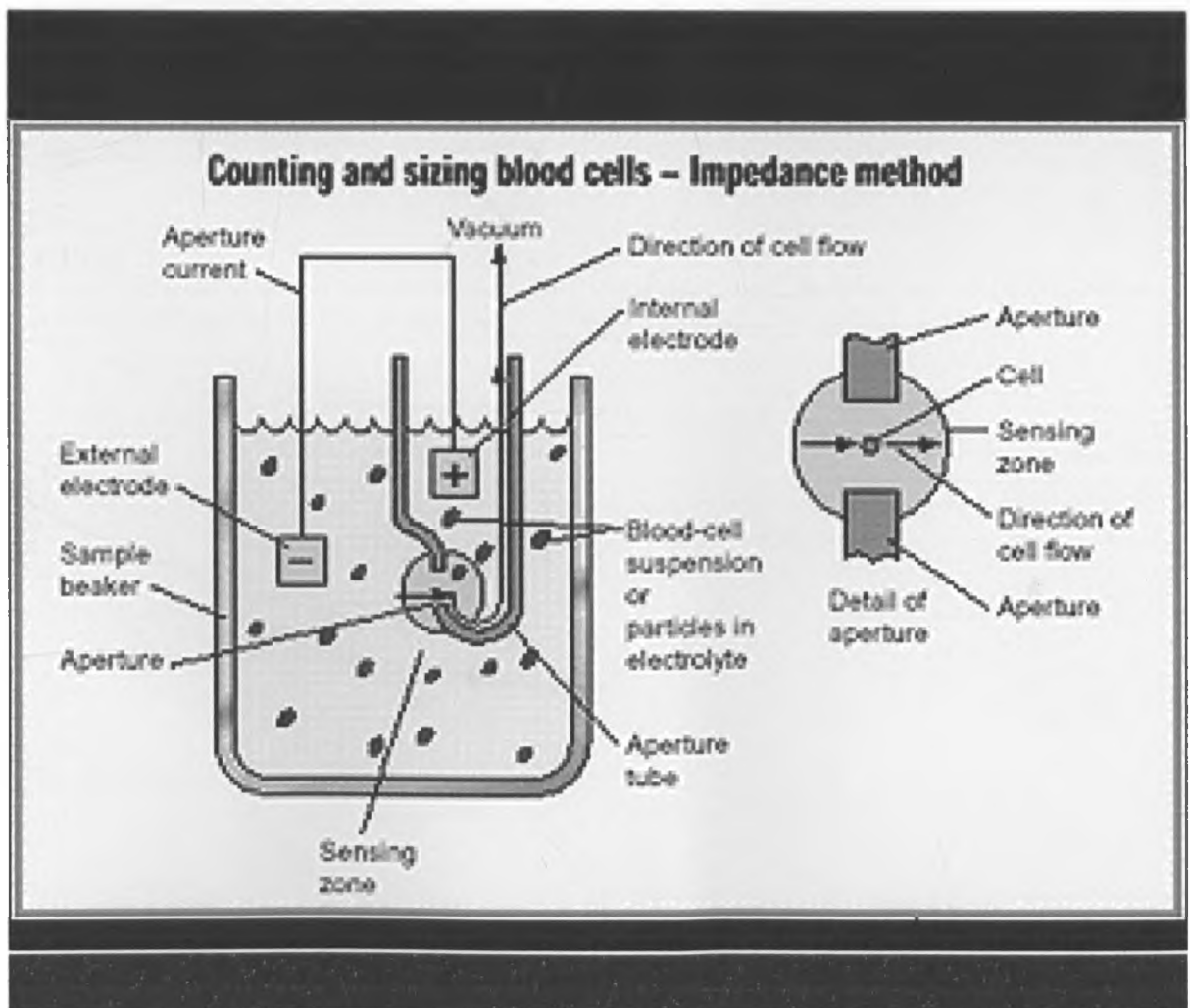
APPENDIX V: Method for blood count quality control sample preparation

1. Collect a unit of human blood into CPD or ACD anti-coagulant.

2. The subsequent procedure is to be carried out no later than one day after collection,

3. Filter the blood through blood transfusion recipient set into a 500ml glass bottle

4. Add 1ml of fresh 40% formaldehyde. Mix well by inverting and then leave on a roller mixer for 1 hour.

5. Leave at 4^0 C for 7 days, mixing by inverting a few times each day. At the end of this period of storage, mix well on a roller mixer for 20 minutes and then, with constant mixing by hand, dispense in 2ml volumes into sterile containers.

Ref: KNH-ERC/ 01/ 4989

5th December 2007

Dr Muturi Charles
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Muturi

RESEARCH PROPOSAL: "COMPARATIVE ASSESSMENT OF AUTOMATED AND MANUAL WHITE BLOOD
CELL COUNTS AT K.N.H HAEMATOLOGY LABORATORY"   (P205/07/2007)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has
reviewed and **approved** your revised research proposal for the period 5th December 2007 –
4th December 2008.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the
deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each
batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research
findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research
study so as to minimize chances of study duplication.

Yours sincerely

PROF C. KIGONDU
AG. SECRETARY. KNH-ERC

c.c.   Prof. K.M. Bhatt, Chairperson. KNH-ERC
          The Deputy Director CS, KNH
          The Dean, School of Medicine, UON
          The Chairman, Dept. of Human Pathology, UON
          Supervisors: Prof. F.K. Abdallah.Dept.of Haematology & Blood Transfusion, UON
                            Prof. O.W. Mwanda, Dept. of Haematology & Blood Transfusion, UON