Factors Impeding interaction process in EFL Classes:
A Case Study of Secondary Schools- *Hassahiesa* Locality, Gezira State, Sudan

Safa Elsiddig Muhammed Ahmed Saeed

B.A in English Language
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Department of Foreign Languages
Faculty of Education – *Hasahisa*

March, 2015
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Supervision Committee:

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<td>main Supervisor</td>
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Factors Impeding Interaction Process in EFL Classes.  
(A case Study of Gezira state, Secondary Level) - Gezira State/Sudan  

ABSTRACT

Interaction in EFL classes has been taken as a problematic area in language teaching and learning. This study is concerned with the factors that impede interaction process in EFL classes. This study aims at achieving a number of objectives. It aims at investigating difficulties encountered by students in communication process at secondary level. It also aims at proving that group work increases EFL learners’ communication. It also aims to examine the effects of seating arrangement on learners’ communication. Finally, the study aims to explain that social factors affect learners’ communication. The study is designed to test four hypotheses. Learners’ level of language influence classroom communication, group work increases EFL learners’ communication; seating arrangement affects learners’ communication and social factors affect EFL learners to communication. The study adapts the descriptive analytical approach. The data collected by means of questionnaire from 50 teachers of secondary school level. Then it was analyzed by the SPSS program. The analyses lead to a number of findings. The most important ones are that learners’ level of language influences classroom communication, group work increases EFL learners’ communication, seating arrangement affects learners’ communication and social factors affect EFL learners to communication. Based on these findings, the study recommends using group work to increase EFL learners’ communication. Arrangement of classroom improves teaching and learning chances of communication. Teachers should reduce social factors that decrease EFL communication. Teachers should apply CLT methodology in EFL teaching. EFL teachers should present a communicative language. Teachers should create a positive atmosphere to make classroom a more pleasant place and motivating for learning. Also class size should be reduced so that learners can have opportunities to interact. School authorities should involve students' families in the follow up process.
حقائق تحد من تواصل الطلاب الدراسي داخل الفصل باللغة الإنجليزية كلغة أجنبية

تتناول الدراسة الحقائق التي تقلل من تواصل الطلاب في التواصل والتفاعل مع الأنشطة الفصلية في اللغة الإنجليزية كلغة أجنبية. تهدف الدراسة إلى بحث الصعوبات المتعلقة بالتواصل الفصلي باللغة الإنجليزية في الفصل. هذه الدراسة أيضًا تؤكد أهمية العمل الجماعي في تحسين التفاعل الفصلي. استخدم الباحث المنهج التحليلي الوصفي في الدراسة كما استخدم الاستبانة لجمع المعلومات من (50) معلم لغة إنجليزية في المرحلة الثانوية. تم تحليل الاستبانة إحصائياً بنظام (SPSS) بعد تحليل الإستبانة أفادت النتيجة بالأتي:

- مستوى الطلاب اللغوي يؤثر على التفاعل الفصلي.
- العوامل الاجتماعية تؤثر على تفاعل الطلاب داخل الفصل.
- العمل الجماعي يعزز التفاعل الفصلي.
- العمل الجماعي داخل الفصل كما توصي المعلم بإستخدام لغة جيدة في عرض المادة الدراسية.
- توصي المعلم بإتباع منهج دراسي جيد ومحفز للطلاب. كما توصي المعلم بإنشاء وضيطة بيئة تدريسية جيدة.
- توصي الدراسة بالحد من زيادة عدد الطلاب المعيق للتواصل في الفصل.
DEDICATION

To my Husband and my Family
ACKNOWLEDGEMENTS

I am heartily thankful to my main supervisor, Dr. Abdul Gadir Muhmmed Ali, whose encouragement, guidance and support from the initial to the final level enabled me to develop and understanding of the subject. Also, word alone cannot express the thanks I owe to my co-supervisor Dr Yosuf A.Nugud, the department foreign language for their encouragement and assistance.

Lastly, I offer my regards to my family and all of those who supported me during the completion of this work.
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Chapter one

Introduction:

1.1 General Introduction:

The average human adult has more than 5 liters of blood in his or her body. Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug in a damaged blood vessel to prevent blood loss (Bruce Alberts, 2002). The methods used to determine the WBC values are important since the assessment of white blood cells counts play an important role in diagnosis and treatment of patients. These require results that are reliable and accurate. In addition the WBC values provided valuable information about the blood and the bone marrow, which is the blood forming tissue. 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 treatment and monitoring of treatment especially to determine the effects of chemotherapy and radiation therapy on blood cells. The methods used to obtain manual WBC values include total counts estimation by both Neubauer chamber and peripheral blood film. The latter methods is also used for the manual 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 blood cell counts only. In recent times, many centers have adopted the automated methods due to a variety of reasons (Muturi, 2008).

Quality becomes one of the most important targets to all people responsible for the administration and development in all aspects of life, specially the medical field. Quality assurance is process oriented and QC is product oriented. QA aims at ensuring, for both laboratory staff and clinicians, that the data provided are relevant and reliable, thus improving trust in laboratory results.

Quality assurance (QA) should include, besides internal quality control (IQC) and external quality assessment (EQA), all elements of its extended concept: these include clinical specimen
handling, preservation, storage, transport, identification and data processing (including reporting of results, recording and charting, interpretation of results and feedback (EL-Nageh et al., 2002).

Internal quality control [IQC] ensures that factors determining the magnitude of uncertainty do not change during the routine use of an analytical method over long periods of time.

IQC is conducted by inserting one or more control materials into every run analysis. The control materials are treated by an analytical procedure identical to that performed on the test materials (Michael, 2010).

External quality control [EQC] is used to describe a method that allows for comparison of laboratory testing to a source outside the laboratory. This comparison can be performance of a peer groupe of laboratories or to the performance of reference laboratory (ISO 5725-1:1994).

1.2 Justifications:

Automated hematological analyzer becomes widely used in laboratories but many labs still work on manual methods. The manual methods are still gold standard in haematology lab if done under quality. Any hematological Analyzer must be evaluated and assessed its accuracy and precision.
1.3 Objectives:

1.3.1 General objective:

To assess Diagnostic performance of Manual, Sysmex and Mindray in measurement of WBCs in Wad Medani City.

1.3.2 Specific objectives:

1. To compare white blood cells count by manual and automated methods.

2. To estimate Repeatability and Reproducibility for manual and automated methods.

3. To estimate sensitivity, specificity for manual and automated methods.

4. To correlate age groups, sex and Marital status for manual and automated methods.
Chapter two

Literature review:

2.1 Laboratory quality control:

Laboratory quality control is designed to detect, reduce and correct deficiencies in a laboratory internal analytical process prior to the release of patient results, in order to improve the quality of the results reported by the laboratory. QC is a measure of precision or how well the measurement system reproduce the same result over time and under varying operating condition. Interpretation of QC data involves both graphical and statistical methods. It is most easily visualized using a Levey–Jenning chart. The dates of analyses are plotted along the X-axis and control values are plotted on the Y-axis. The mean and one two and three standard deviation limits are also marked on the Y-axis. Inspection the pattern of plotted points provides a simple way to detect increased random error and shifts or trends in calibration (Tietz, 1987, Grant, 1988).

2.1.1 Quality laboratory processes (QLPs):

This includes analytical processes as well as general policies, practices and procedures that define how all aspect of the work will be done (Westgard, 1986).

2.1.2 Quality assessment (QA):

It is concerned with broader measures and monitors of laboratory performance, such as turnaround time, specimen identification and test utility (Juran et al., 1951).

2.1.3 Quality control:

QC emphasizes statistical control procedures and also includes reagent and standard checks, linearity checks … etc (ISO 5725-1:1994).

2.1.4 Precision:

Is the degree of similarity among independently measurements of the same quantity without reference to the known or true value (EL-Nageh et al., 2002).
2.1.5 Accuracy:

It is the extent to which the measured value of quantity agree with the accepted value for that quantity, i.e. the closer to the actual value, the more accurate (EL-Nageh et al., 2002).

2.1.6 Sensitivity:

Is a measure of the incidence of positive results in patient known to have a condition that is True positive (TP) (EL-Nageh et al., 2002).

2.1.7 Specificity:

Is a measure of the incidence of negative results in persons known to be free of disease that is True negative (TN) (EL-Nageh et al., 2002).

2.1.8 Repeatability:

Is refers to the variation in repeat measurements made on the same subject under identical conditions over a short period of time (Bartlett et al., 2008).

2.1.9 Reproducibility:

Is refers to the variation in measurements made on a subject under changing conditions over a period of time (Bartelett et al., 2008).

2.1.10 Control:

This is a sample that is chemically and physically similar to unknown specimen (Westgard, 1986).

2.1.11 Standard:

It is something established for use as a rule or basis of comparison in measuring or judging capacity, quantity, content extent, value, or quality (ISO 5725-1:1994).

2.1.12 Quality:

The totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs (ISO 5725, 1994).
2.1.13 Quality system:

This is the organizational structure, responsibilities, procedures, processes, and resources for implementation of quality management (ISO 5725-1:1994).

2.2 Leukopoiesis:

*White blood cells (WBCs) are a heterogeneous group of nucleated cells that can be found in circulation for at least a period of their life. Their normal concentration in blood varies between 4.000 and 11.000 per microliter. They play a most important role in phagocytosis; immunity and therefore in defense against infection. Leukocytes are usually divided into granulocytes, which have specific granules; are divided into neutrophils, eosinophils and basophils. A granulocyte, which lack specific granules; are divided into lymphocytes and monocytes. All WBCs are produced and derived from multipotent cells in the bone marrow known as haematopoietic stem cells: they contain nuclei and usually organelles, which distinguished them from the other blood cells the a nucleated red blood cells (RBCs) and platelets. In the bone marrow there is a 4:1 ratio, the M:E ratio indicating that four myeloid, or white cells are produced for one erythroid cell. Daily production of white cells is 1.5 billion. Transite from the bone marrow to the peripheral circulation takes place only after white blood cells have been held in storage pool of the bone marrow. While white cells journey spend only hours in circulating blood. The pluripotent stem cell give rise to the myeloid stem cell and the lymphoid stem cell. Through a serious of intervention from interleukins (chemical stimulators) and growth factors a CFU-GEMM is structured to give rise to granulocytes, erythrocytes, monocytes and macrophages. Curiously, megakaryocyte, eosinophils and basophils have their own CFU:CGU-Meg and CFU- Eosinophil / basophil. Lymphocytes originated not only from the bone marrow but also from the thymus and thus, they have a disinctive place on the haemopoietic maturation (Betty,2007).

2.2.1 Leucocytes stages and functions:

The white cell series encompasses those cells that are distinguished by their granules and those that are a granular. In all, there are five maturation stages for neutrophils, four for eosinophils
and basophils, and three each for monocytes and lymphocytes. Key features in distinguishing immature and mature stages of any of these cells are: cell size, nucleus–cytoplasm ratio (N:C), chromatin pattern, cytoplasmic quality, and presence of granules (Hoffbrand et al., 2006).

2.2.2 The granular cells:

2.2.2.1 Neutrophils:

Development: The stages of maturation for the neutrophilic series from least mature to most mature are:
- Myeloblast.
- Promyelocyte or progranulocyte.
- Myelocyte.
- Metamyelocyte.
- Band.
- Segmented neutrophil (Betty, 2007, Ogawa, 1986).

*Segmented neutrophils “also called polymorphnuclear neutrophil leukocytes (PMNs) have a nucleus divided into multiple distinct lobes connected by thin strands of chromatin. The cytoplasm has fine granules that stain lightly with the usual blood stain. Polys normally comprise 50 to 70% of total WBCs. *Band neutrophils” also called stabs” have a horseshoe-shaped nucleus, without the distinct lobes of polys. They are an earlier stage than segmented neutrophils but are fully functional. Bands normally represent ~2 to 6% of all WBCs; the number of bands increases with acute stress or infection (William, 2002). In women 2-3% of neutrophils show an appendage at the terminal nuclear segment. This “drumstick” is about 1.5 mm in diameter and is connected to the nucleus by short stalk. It represents the inactive X chromosome and corresponds to the Barr body of buccal cells (Dacie and Lewis, 2011).

2.2.2.1.1 Functions of neutrophils:

In normal adults, neutrophils account for more than half the circulating leukocytes. They are the main defense of the body against pyogenic bacterial infections. About half the neutrophils in the blood, circulate forming circulatory pool. The other half, marginate along the walls of blood vessels and in capillaries, forming the marginating pool. Increased numbers of neutrophils in the
marginating pool may explain the lower circulating neutrophil counts frequently found in African people. Neutrophils have a short life-span. After circulating in the blood for 6-10 hours they pass into the tissue where as highly mobile phagocytes they are important in defending the body from infection. They mobilize and migrate to sites of infection or inflammation, chemically attracted by substance released by bacteria, complement components, damaged tissue, and other leukocytes process called chemotaxis. Neutrophils have receptors for IgG antibody and complement (c3b) and are therefore able to recognize, phagocyte and kill bacteria coated with IgG and c3b (Cheesbrough, 2006).

2.2.2.2 Eosinophils:

Development: Eosinophils like neutrophils originated, differentiate, and mature in the bone marrow. They are a little larger than neutrophils, 12-17 mm in diameter; have two nuclear lobes or segments, and the cytoplasm is packed with distinctive spherical gold/orange granules. The underlying cytoplasm, which is usually obscured by granules, is pale blue (Dacie and Lewis, 2011).

2.2.2.1 Functions of eosinophils:

Eosinophils enter the blood from the bone marrow. They circulate for about 8 hours after which they enter the tissues. They are found mainly in the skin, gastrointestinal tract and lungs where they are involved in hypersensitivity reactions, e.g. asthma, hay fever, eczema. Also it’s important in parasitic helminth immune responses in which IgG and IgE antibodies are produced (Cheesbrough, 2006).

2.2.2.3 Basophils:

Development: Basophils like neutrophils and eosinophils, originate, differentiate and mature in the bone marrow. Basophils are the rarest (1%) of the circulating leukocytes. Their nuclear segments tend to fold up to each other, resulting in a compact irregular dense nucleus resembling a closed lotus flower. The distinctive, large variably sized, dark blue or purple granules of the cytoplasm often obscure the nucleus, they are rich of histamine, serotonin and heparin. Basophils tend to degranulate, leaving cytoplasmic vacuoles (Hoffbrand et al., 2006, Saladin, 2012).
2.2.2.3.1 Functions of basophils:

Basophils circulate only in the blood. They are not found in tissues (mast cells are tissue equivalents to basophils). Basophils bind to IgE on their surface and are involved in anaphylactic, hypersensitivity and inflammatory reactions. Basophils interact with eosinophils and macrophages in allergic reactions (Dacie and Lewis, 2011).

2.2.3 The A granular cells:

2.2.3.1 Monocytes:

Monocytes are the largest of the circulating leukocytes 15-18mm in diameter. They have bluish–grey cytoplasm that contains variable numbers of fine reddish granules. The nucleus is large and curved, often in the shape of a horseshoe, but it may be folded or curried (Greer et al., 2003).

2.2.3.1.1 Functions of monocytes and macrophages:

Monocytes pass from the bone marrow into the blood circulation. Within 2-3 days they reach the tissues where they develop into macrophages, becoming fixed tissue macrophages in the spleen, liver (Kupffer cells), lymph nodes connective tissues, and central nervous system, and free macrophages in lung alveoli, peritoneum and inflammatory granulomas. Macrophages form the mononuclear phagocytic system, i.e. reticuloendothelial system (RE). The RE system is involved in the destruction of bacteria, viruses, fungi, protozoal parasites, and malignant cells. Macrophages present antigens to T lymphocytes, and regulate many T and B cells activities. When activated, macrophage synthesize and secrete cytokines (e.g. interleukins, tumor necrosis factors, GM-CSF) which are involved in the activation of lymphocytes, the inflammatory process particularly chronic inflammation, cell-mediated immune responses and haematopoiesis (Cheesbrough, 2006).

2.2.3.2 Lymphocytes:

Development: The lymphocytic series is distinctive in its presentation and function. In contrast to most other white cells, which are derived solely from the bone marrow, lymphocytes are derived from two locations. The primary lymphoid organs are the bone marrow and thymus, The secondary lymphoid organs are the spleen, lymph nodes, peyer’s patches of the gastrointestinal tract, and the tonsils (Dacie, 2011, Siekmerier et al., 2001).
2.2.3.2.1 Lymphocytes are the second most common type of leukocytes in adult:

*Resting lymphocyte: Are usually small (7-10µm), with a dark round to oval nucleus and scant amounts of pale blue cytoplasm.

*Reactive “atypical” lymphocytes: A minority of lymphocytes are larger, with more abundant pale blue cytoplasm and larger nuclei with less condensed chromatin.

*Large granular lymphocytes: A small number of lymphocytes in normal blood are slightly larger than resting lymphocytes, with reddish-purple (azurophilic granules). This appearance generally corresponds to natural killer (NK) cells. Functionally, there are two main types of lymphocytes: B cells, are the primary effectors of humoral (antibody-mediated) immune system. T cells, are the effector of (cell-mediated) immunity (William, 2002).

2.2.3.2.2 Functions of lymphocytes:

Lymphocytes are important cells in specific immune responses. CD4 helper T lymphocytes secrete cytokines which stimulate B lymphocytes to develop into antibody-producing plasma cells. They also help to activate CD8 cytotoxic T lymphocyte, participate in delayed hypersensitivity reactions with macrophages, and modulate cellular immune response. CD4 T cells respond to recognize antigen bound to MHC (major histocompatibility complex) class 1 protein. CD8 cytotoxic lymphocytes: produce toxins which destroy tumor cell and cells infected with viruses and other microorganisms. They respond to antigen bound to MHC class 1 (Cheesbrough, 2006).

2.3 Factors affecting Total leukocytes variables:

Gender, age, pregnancy, labour, postpartum, menstruation, menopause, exercise, cigarette smoking, obesity etc….(Bain, 2006).

2.4 White blood cell disorders:

2.4.1 Quantitative changes in white cells:

Because WBCs have such a short time span in the peripheral circulation, alterations either in the quantity of the quality of a particular cell can be quite dramatic. With the normal white cells ranges for adults and children as a benchmark, any increase or decrease in a particular type of
cell signals the body’s unique response to assaults of any kind, infection, inflammation, chronic disease, parasitic infestations, etc ……

If a cell line is increased, the suffix used to designated an increase is “osis” or “philia” such as “basophilia” and “lymphocytosis”, if a cell line is decreased, the suffix used to designate a decrease is “penia”, such as “lymphopenia” (Betty, 2007).

2.4.2 Qualitative changes in white cells:
Qualitative changes of white cells take place either in the cytoplasm or in the nucleus. These changes are classified as either inherited or acquired. Acquired defects are seen with much greater frequency than inherited abnormalities. Once a patient has developed an increased white count, toxic changes of white cells usually occur due to stress during maturation and as a result of activity in the circulation or tissue (Betty, 2007).

2.5 Laboratory diagnosis:
2.5.1 White blood cell counts:
White cells can be counted manually in or with automated counters. A manual white cell count (WBC) is performed after diluting an aliquot of blood in diluents that lyses red cells and stains the nuclei of white cells.

White cells are counted microscopically in a haemocytometer with chambers of known volume (Bain, 2006).

2.5.1.1 Manual white cell count:
Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells (not the nucleated red cells), leaving the white cells to be counted microscopically using an improved chamber “haemocytometer” and the number of WBCs per liter of blood calculated (Cheesbrough, 2006). Manual leukocytes counts have more inherent error, with CVs ranging from 6.5% in cases with normal or increased white cell counts to 15% in cases with decreased white cell counts (Greer et al., 2003).

2.5.1.1 Sources of errors in manual WBCs counts:
Incorrect measurement of blood due to poor technique.
Using unsuitable anticoagulated blood, not mixing the blood sufficiently or not checking the sample for clots.

Not using a haemocytometer cover glass.

Not correcting a count when the sample contains many nucleated RBCs.

Using too intense a light source (Turgon, 1999).

2.5.1.2 Automated white blood cells count:

White cells can be counted by aperture impedance or light scattering techniques. Automated impedance counters, this type of analyzer, (which includes the Sysmex), enumerates cells in a small aperture by measuring changes in electrical resistance as the cell passes through the orifice. A constant current passes between two platinum electrodes on either side of the orifice. The diluent that suspends the cells is more electrically conductive than are the cells. Hence, as each cell passes through the orifice, there is a momentary decreased in electrical conductance so that an electrical impulse is generated and recorded electronically. The drop in voltage is proportional to cell size, allowing average cell size to be determined simultaneously (Burnett, 2005).

2.5.1.2.1 Sysmex XP 300 Haematology analyzer and principle:

The sysmex XP 300 (Sysmex corporation, Kobe, Japan) is an automated blood cell counter intended for in vitro diagnostic use in clinical laboratories.

It is a compact, fully automated haematology analyzer with simultaneous analysis of 18 parameters in whole blood and the instrument has been proven to provide accurate and reliable, the test was performed as stand in the manufacturers manual.

Sheath flow DC detection is the basic principle for measuring the number of cells and distinguishing their types by size (Sysmex, 2014).

2.5.1.2.2 Mindray BC-6800 and principle:

BC- 6800 Auto Haematology Analyzer is a quantitative, automated haematology analyzer for in vitro Diagnostic use in clinical laboratories, it provides Complete blood count, leukocytes 5-part Differential, haemoglobin concentration Measurement, Reticulocyte and Nucleated Red Blood Cell Measurement for blood samples, as well as Body fluid analysis.
Sheath flow impedance method, laser scatter and SF cube cell analysis technology (3D analysis using information from scatter of laser light at two angles and fluorescence signals) for cell differentiation and counting and the colorimetric method for HGB measurement (Mindray, 2013).

**2.5.1.2.3 Sources of error in Automated WBC count:**

Errors occur in automated WBCs are usually detected because of instrument flags and improbable results for the WBC or other measurements, or by abnormalities detectable on instrument scatter plots or histograms.

*Some causes of a falsely high WBC:*

Presence of NRBCS.

Numerous giant platelets.

Non lysis of red cells.

Uraemia.

Abnormal haemoglobins (e.g. AS, SS).

Malarial parasites.

Post spleenectomy.

Liver disease.

*Some causes of a falsely low WBC:*

Cell lysis caused when blood is more than 3 days old.

Storage at room temperature for 24 hours or more.

Leukocyte and platelet aggregation (Dacie, 2011).

**2.6 Previous studies:**

In Ozalla, Enugu state, Nigeria, Samuel *et al.*, (2010), from 60 subjects 3 mls of venous blood sample was collected from each subject into tri-potassium EDTA for the analysis of haematological parameters using the Sysmex KX-21N and manual methods. After the analysis
showed that WBCs demonstrated highly statistically significant difference (P.value<0.001) and correlate positively when both methods were compared.

In Iraq, during the period of comparative cross sectional study Kaream et al.,(2016), from 52 cases of blood sample were collected into tri-potassium EDTA admitted to hospital for the analysis of WBCs by Sysmex XP -300 in the haematological department laboratory performance manual method in the same time, showed that WBCs demonstrated highly statistically significant difference (P.value=0.001).
Chapter Three

Materials and Methods

3.1 Study design:
This was an analytical cross sectional study aimed to compare white blood cells count among healthy Sudanese adults in Wad Medani city.

3.2 Study area and duration:
This study was done in Wad Medani City from July to August 2016. The Gezira is a well-populated area suitable for agriculture. Wad Medani is the capital of the state. The name comes from the Arabic word for island.

3.3 Study population:
Adults people in Wad Medani according to inclusion and exclusion criteria.

3.4 Sampling and sample size:
The research was used stratified random sampling with proportional to size technique.

3.6 Study criteria:
3.6.1 Inclusion criteria:
*Sudanese nationality.
*Both gender.
*Age group (20 and above 40 years).

3.6.2 Exclusion criteria:
*History of any chronic illness from which the individual is still suffering, e.g. hypertension, diabetes.
*Common extraneous influences affecting parameters under study e.g. cigarette smoking, alcohol intake.
* modified physiological states that cause changes in parameters under study, e.g. pregnancy, fasting individuals, etc.

3.7 Data collection tool:

The data was collected by using a questionnaire (Appendix 3). A questionnaire was designed to include all needed informations.

3.8 Data analysis:

Data was analyzed by Microsoft Excel sheet, Medical Calculator computer program (Medcalc version 16.8) and SPSS version 16.

3.9 Ethical Consideration:

Ethical approval of this study was obtained from the ministry of health in Gezira state. The specimens and information were collected from individuals under privacy and confidentiality and were not used for any purposes rather than this study.

3.10 Materials:

The following materials were utilized in this study:

- Lab coat.
- Gloves.
- Cotton.
- Tourniquet.
- Syringes.
- K3 EDTA container tube.
- Electrical mixer device.
- Chamber.
- Sysmex XP 300 (Serial number no: A3334).
- Mindray BC-6800.
- Diluent reagent.
- Lysis reagent.
- Detergent.
- Microscope.
- Frosted end slides.
- Capillary pipette.
- Spreader.

3.11 Methods:

3.11.1 Sample collection:
Venous blood was drawn from anticubital vein using aseptic technique. 3 mls of venous blood were withdrawn by using evacuated blood collection tube containing K3 EDTA; the blood was mixed with anticoagulant by inverting the tubes several times, and the tubes was labeled with an identifying number.

3.11.2 Manual Method:

3.11.2.1 Principle:
A sample of whole blood is mixed with a weak acid solution (acetic acid) that lyses red blood cells but preserves leukocytes, the diluted blood is added to the hemocytometer chamber. Cells are allowed to settle for 10 minutes before leukocytes counted.

3.11.2.2 Reagents:
- 2% acetic acid. Add 2 ml glacial acetic acid to 100 ml volumetric flask. Dilute to the mark with distilled water.
- 1% hydrochloric acid. Add 1 ml hydrochloric acid to 100 ml volumetric flask. Dilute to the mark with distilled water.
3.11.2.3 Procedure:

Make a 1 in 20 dilution of blood by adding 0.1 ml of well mixed blood (Lake of adequate mixing is a major source of error) to 1.9 ml of lysing fluid (2% (20 ml /l) acetic acid coloured pale violet with gentian violet) in a 75×10 mm glass or plastic tube, after that mix the diluted blood in mechanical mixer or by hand for at least 2 min by tiling the tube through an angle of about 120 combined with rotation, thus allowing the air bubble to mix the suspension. Fill a clean dry counting chamber, with its cover glass already in position, without delay. This is simply accomplished with the aid of a Pasteur pipette or a length of stout of capillary glass tubing which has been allowed to take up the suspension by capillarity. Care should be taken that the counting chamber is filled in one action and 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, but not much longer. The bench must be free of vibrations and the chamber not exposed to direct sunlight or other sources of heat. The cover glass should be of such a size that when placed correctly on the counting chamber the central ruled areas lie in the centre of the rectangle to be filled with the cell suspension.

If any of the following filling defects occur, the preparation must be discarded and the filling procedure repeated using another clean dry chamber:

* Overflow into moat.
* Chamber area incompletely filled.
* Air bubbles anywhere in chamber area.
* Any debris in chamber area.

3.11.2.4 Calculation:

White blood cell count per liter (WBC/l) =

No. of cells counted / volume counted (µl)×Dilution×10

Thus, if N cells are counted in 0.1 µl, then the WBC/l is

N ÷ 0.1×20×10^6

e.g. if 115 cells are counted, the WBC IS 115×200×10^6/l = 23×10^9 /l
3.11.3 White blood cells by XP 300:

3.11.3.1 Principle:

Sheath flow DC detection is the basic method for measuring the number of cells and distinguishing their types by size.

3.11.3.2 Reagents:

- CELLPACK: Is a diluents used to dilute aspirated analysis samples in order to measure an RBC count, WBC count, haemoglobin concentration and platelet count.

- STROMATOLYSER-WH : Is a reagent that lyses RBC for accurate WBC count determination, WBC tri-modal size distribution analysis and haemoglobin level measurement.

- CELLCLEAN : Is a strong alkaline detergent used to remove lyse reagents, cellular residuals and blood proteins remaining in the hydraulics of the instrument.

- EIGHTCHECK-3WP : Is control blood for testing the precision and accuracy of haematology analyzers.

See Appendix(2).

3.11.3.3 Procedure:

- Blood is aspirated from the sample probe into the SRV.

- 6 µl of blood measured by the SRV is transferred to the WBC TD chamber along with 1.994 ml of diluent. At the same time, 1.0 ml of WBC/HGB lyse is added to prepare 1:500 dilution sample.

- When the solution is made to react in this status approximately 10 seconds, RBC is haemolyzed and platelets shrink, with WBC membrane held as they are.

- 500 µl of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
3.11.4 White blood cells by Mindray BC-6800:

3.11.4.1 Principle:
Sheath flow impedance method, laser scatter and SF cube cell analysis technology (3D analysis using information from scatter of laser light at two angles and fluorescence signals) for cell differentiation and counting.

The colorimetric method for HGB measurement.

3.11.4.2 Reagents:
See Appendix (1)

3.11.4.3 Procedure:
BC-6800 adopts the SF cube cell analysis technology to recognize and detect the immature cells in the blood accurately besides doing WBC 5-part differentiation. Based on the analysis of the BASO channel scattergram and Bas region, the analyzer gets the White blood cell count (WBC) and Basophil number (Bas#). The Basophil percentage is then calculated. Based on the analysis of DIFF channel scattergram and lym region, Neu region (IMG region), Mon region and Eos region, the analyzer get the percentage of lymphocyte (Lym %), neutrophil (Neu%), monocytes (Mon%), Eosinophil (Eos%), and immature granulocytes (IMG%, which is included in Neu%). The number of lymphocyte (Lym%), neutrophils(Neu%), monocytes(Mon%), eosinophils(Eos%) and immature granulocytes (IMG#, which is included in Neu#) are then calculated based on the sub-population percentages together with the White blood cell count got from the Bas channel. The cells numbers are all expressed in $10^9/\text{L}$

*WBC count=Sum of all particles in BAS channel except those in Ghost region.
Chapter Four

Result

The study was conducted to estimate repeatability and reproducibility of white blood cells count, 75 samples were included, 31 (47%) of them were males, while 44 (53%) were females, their age between (20-56) years, each sample was repeated three time by using Sysmex XP-300, Mindray BC-6800 and Manual method.

There was no significant difference in repeatability of WBCs by Manual method.

Table 4.1  Repeatability of White Blood Cell count using Manual method

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual reading 1</td>
<td>5.1707</td>
<td>0.2137</td>
<td>4.7449 to 5.5964</td>
<td>0.206</td>
</tr>
<tr>
<td>Manual reading 2</td>
<td>5.0293</td>
<td>0.2019</td>
<td>4.6271 to 5.4316</td>
<td>0.210</td>
</tr>
<tr>
<td>Manual reading 3</td>
<td>4.9560</td>
<td>0.1920</td>
<td>4.5734 to 5.3386</td>
<td>0.210</td>
</tr>
</tbody>
</table>

There was significant difference in repeatability of WBCs by Sysmex XP-300 P.value (0.045)

Table 4.2  Repeatability of White Blood Cell count using Sysmex XP-300

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sysmex reading 1</td>
<td>6.0093</td>
<td>0.1993</td>
<td>5.6122 to 6.4065</td>
<td>0.045</td>
</tr>
<tr>
<td>Sysmex reading 2</td>
<td>5.9800</td>
<td>0.1974</td>
<td>5.5867 to 6.3733</td>
<td>0.045</td>
</tr>
<tr>
<td>Sysmex reading 3</td>
<td>5.9627</td>
<td>0.1976</td>
<td>5.5689 to 6.3564</td>
<td>0.045</td>
</tr>
</tbody>
</table>
There was no significant difference in repeatability of WBCs by Mindray.

**Table 4.3 Repeatability of White Blood Cell count using Mindray BC-6800**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mindray reading 1</td>
<td>5.8680</td>
<td>0.1991</td>
<td>5.4713 to 6.2647</td>
<td>0.897</td>
</tr>
<tr>
<td>Mindray reading 2</td>
<td>5.8573</td>
<td>0.1982</td>
<td>5.4623 to 6.2523</td>
<td>0.797</td>
</tr>
<tr>
<td>Mindray reading 3</td>
<td>5.8507</td>
<td>0.2072</td>
<td>5.4378 to 6.2636</td>
<td>0.799</td>
</tr>
</tbody>
</table>

There was significant difference in reproducibility of WBCs by (Manual, Sysmex and Mindray). P.value(<0.001).

**4.4 Correlation of White Blood Cell count between all methods**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean_of_manual_method</td>
<td>5.0520</td>
<td>0.1901</td>
<td>4.6733 to 5.4307</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean_of_Sysmex_method</td>
<td>5.9840</td>
<td>0.1978</td>
<td>5.5899 to 6.3781</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean_of_Mindray_method</td>
<td>5.8587</td>
<td>0.2004</td>
<td>5.4594 to 6.2580</td>
<td>&lt;0/001</td>
</tr>
</tbody>
</table>

There was no significant difference of gender and WBCs by (Manual, Sysmex and Mindary).

**4.5 Correlation of White Blood Cell count and Gender**

<table>
<thead>
<tr>
<th></th>
<th>Male N = 31 Means of WBCs ± SD</th>
<th>Female N = 44 Means of WBCs ± SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Method</td>
<td>5.4 ± 1.8</td>
<td>6.2 ±1.7</td>
<td>0.612</td>
</tr>
<tr>
<td>Sysmex Method</td>
<td>4.7 ± 1.6</td>
<td>5.3 ± 1.6</td>
<td>0.746</td>
</tr>
<tr>
<td>Mindray Method</td>
<td>5.6 ± 1.7</td>
<td>6.3 ± 1.7</td>
<td>0.710</td>
</tr>
</tbody>
</table>
There was no significant difference of status and WBCs by (Manual, Sysmex and Mindray)

### 4.6 Correlation of White Blood Cell count and Marital Status

<table>
<thead>
<tr>
<th></th>
<th>Single N = 46 Means of WBCs ± SD</th>
<th>Marriage N = 29 Means of WBCs ± SD</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Method</td>
<td>5.8 ± 1.7</td>
<td>5.9 ± 1.7</td>
<td>0.969</td>
</tr>
<tr>
<td>Sysmex Method</td>
<td>5.1 ± 1.8</td>
<td>5.0 ± 1.5</td>
<td>0.374</td>
</tr>
<tr>
<td>Mindray Method</td>
<td>6.0 ± 1.8</td>
<td>6.0 ± 1.6</td>
<td>0.724</td>
</tr>
</tbody>
</table>

There was no significant difference of age group and WBCs by (Manual, Sysmex and Mindray)

### 4.7 Correlation of White Blood Cell count and age group

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Method</td>
<td>20 - 29 Years</td>
<td>25</td>
<td>5.070</td>
<td>1.7797</td>
</tr>
<tr>
<td></td>
<td>30 - 39 Years</td>
<td>25</td>
<td>4.986</td>
<td>1.2874</td>
</tr>
<tr>
<td></td>
<td>40 and above Years</td>
<td>25</td>
<td>5.033</td>
<td>.7082</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>5.052</td>
<td>1.6460</td>
</tr>
<tr>
<td>Sysmex Method</td>
<td>20 - 29 Years</td>
<td>25</td>
<td>6.008</td>
<td>1.8036</td>
</tr>
<tr>
<td></td>
<td>30 - 39 Years</td>
<td>25</td>
<td>5.679</td>
<td>1.3578</td>
</tr>
<tr>
<td></td>
<td>40 and above Years</td>
<td>25</td>
<td>6.708</td>
<td>1.5892</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>5.984</td>
<td>1.7130</td>
</tr>
<tr>
<td>Mindray Method</td>
<td>20 - 29 Years</td>
<td>25</td>
<td>5.869</td>
<td>1.7783</td>
</tr>
<tr>
<td></td>
<td>30 - 39 Years</td>
<td>25</td>
<td>5.629</td>
<td>1.5648</td>
</tr>
<tr>
<td></td>
<td>40 and above Years</td>
<td>25</td>
<td>6.517</td>
<td>1.9555</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>5.859</td>
<td>1.7355</td>
</tr>
</tbody>
</table>
The sensitivity, specificity and AUC was shown: the sensitivity 48.15%, specificity 61.9%, AUC give cut off value less than 70% which reflect the poorness of the test for count WBCs.

**figure 4.1:** ROC analysis between manual method and Sysmex
The sensitivity, specificity and AUC was shown: the sensitivity 92.59%, specificity 28.57%, AUC 60% which beyond the cut off value for the recommended AUC.

**Figure 4.2:** ROC analysis between manual method and Mindray
The sensitivity was shown 49.23% while specificity was 80%, AUC was 64% which reflect the poorness of the test for count WBCs.

**Figure 4.3:** ROC analysis between Mindray and Sysmex
Chapter five

Discussion

Clinical practice involves measuring quantities for a variety of purposes, such as aiding diagnosis, predicting future patient outcomes, and serving as endpoints in studies or randomized trials. Furthermore, haematology analyzers provide quick, more efficient and accurate results in most situations. However, false results related either to platelets or other parameters from complete blood count may be observed in several instances, false low WBCs counts correlate with agglutination in the presence of ethylenediamine-tetra-acetic acid. Despite the sophistication of present day instruments, there is still need to depend on manual techniques for primary calibration. The studied samples represented healthy people who were not suffering from any diseases, when comparing repeatability of WBCs by manual, sysmex and mindray showed no significant differences except by sysmex P.value(0.04) and when relating the means of WBCs count that estimated by manual and automated method for all samples (n= 75) showed highly statistically significant difference P.value (<0.001) this agree with finding seen in study done by Karem Kder et al., in Iraq during the period of comparative cross sectional study, 52 cases of blood sample were collected into tri-potassium EDTA admitted to hospital during February to April 2016 for the analysis of WBCs by Sysmex XP-300 in the haematology department laboratory performance manual method in the same time, showed the WBCs demonstrated highly statistically significant difference P.value (0.001). Samuel O Ike et al., in Nigeria, Ozalla, Enugu state (2010), 3 mls of venous blood sample was collected from each subject into tri-potassium EDTA for the analysis of haematological parameters using the automated and manual methods. After the analysis showed the WBCs demonstrated highly statistically significant difference P.value<(0.001).

White blood cell counts, like all laboratory tests, are subject to both inaccuracy and imprecision. (Bain, 2006) Statistical errors are the main source of error inherent in manual counts, imprecise and labor-intensive but it is easy to perform and need very simple reagents.

In this study when using Area Under the Curve (AUC), to test the sensitivity and specificity of automated versus manual method, the study indicated that Sysmex gave very low sensitivity when compared to manual, this could be related to inability of Sysmex to give accurate total
white blood cell count in low concentration. Like with, the specificity of Mindray is less than the recommended cut off value (70%) when compared to manual, this result was directly related to poorness of Mindray to differentiate between different classes of white blood cells.
Chapter Six

Conclusion and Recommendations

6.1 Conclusion

1. There was no significant difference between repeatability of WBCs by Manual and Mindray.

2. There was significant difference between repeatability of WBCs by Sysmex.

3. There was significant difference between reproducibility of WBCs by all methods.

4. There was no significant difference between the white blood cells count according to (gender, status and different age group).

5. Evaluation of Mindray BC-6800 by ROC curve showed sensitivity 92.59%, AUC 60%, this indicate high sensitivity of Mindray BC-6800.

6.2 Recommendations:

- Pathological samples should be included in large sample size.

- Accuracy and precision must be assessed for manual and automated procedures.

- Periodical maintenance for analyzer.

- Daily calibration for analyzer.

- Mindray BC-6800 should introduced in each lab has over work load.
REFERENCES


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Muturi, Charles Kibicho Mb Chb (UON) (2008), Comparative Assessment of automated and manual white blood cell count at KENYATTA NATIONAL HOSPITAL, NIROBI.


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Appendix (1)

Reagents:
M-68DS diluent
M-68DR diluent
M-68FR dye
M-68LD lyse
M-68FD dye
M-68LN lyse
M-68FN dye
M-68LB lyse
M-68LH lyse
### Appendix (2)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Volume</th>
<th>Storage temp</th>
<th>Usage temp</th>
<th>Shelf life after first opening</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CELLPACK</strong></td>
<td>20 L</td>
<td>1 – 30°C</td>
<td>15 – 30°C</td>
<td>60 Days</td>
<td>Sodium chloride 6.38 g/L, Boric acid 1.0 g/L, Sodium tetraborate 0.2 g/L, EDTA-2K 0.2 g/L</td>
</tr>
<tr>
<td></td>
<td>10 L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>STROMATOLYSER-WH</strong></td>
<td>500 ml × 3</td>
<td>2 – 35°C</td>
<td>15 – 30°C</td>
<td>90 Days</td>
<td>Organic quaternary ammonium salts 8.5 g/L, Sodium chloride 0.6 g/L</td>
</tr>
<tr>
<td><strong>CELLCLEAN</strong></td>
<td>50 ML</td>
<td>1 – 30°C</td>
<td>15 – 30°C</td>
<td>—</td>
<td>Sodium Hypochlorite (available chlorine concentration 5.0%)</td>
</tr>
</tbody>
</table>
Appendix (3)

University of Gezira
College of Graduate studies
Department of haematology


Date: ……………..  ID NO: (……)

Name: ………………………………

Age: 18-28 ( ) 29-38 ( ) 39-56 ( )

Sex: Male ( ) Female ( )

Status: single ( ) married ( )

Contact Number: ………………………

Medical History:
Did you suffer from any disease  Yes ( ) No ( )

If Yes mention……………………………………………………………………………………………………

Did you undergo to surgical operation during the last 12 month  Yes ( ) No ( )

Did you donate or recived blood the last 6 month  Yes ( ) No ( )

Did you infected with malaria during the last month  Yes ( ) No ( )

Are you using any type of medication  Yes ( ) No ( )
If Yes mention………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………

……………………………………………………………………………………………………………………………
Appendix (4)

جامعة الجزيرة
كلية علوم المختبرات الطبية
الدفعة الثانية ماجستير
إقرار مشاركة

الاسم: ........................................................................

سوف يتم أخذ عينة من الدم الوريدي (3) مل بغرض البحث العلمي, كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

أوافق أنا المذكور أعلاه أخذ عينة لإجراء الدراسة.

التاريخ: ................................................................

التوقيع: ................................................................