INTER- LABORATORY VARIABILITY OF HAEMOGLOBIN MEASUREMENTS OBTAINED FROM SELECTED CLINICAL LABORATORIES IN KENYA

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Inter- Laboratory Variability of Haemoglobin Measurements Obtained From Selected Clinical Laboratories in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university**.**

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DEDICATION

I dedicate this thesis to my family, my brothers and sisters for their moral support and encouragement during the course of this study.

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OPERATIONAL DEFINATIONS

Accuracy--------- Is the degree of closeness of measurements of a quantity to that quantity's actual (true) value.

Allowable Total Error(T_AE)---------- A quality requirement that sets a limit for combined imprecision (random error) and bias (inaccuracy, or systematic error) that are tolerable in a single measurement or single test result to insure clinical usefulness.

Analytic Variability---- Random error in a laboratory result whose magnitude depends on the methodology or analyser used.

Bias(inaccuracy)------ Bias is the difference (generally unknown) between a laboratory's average value (over time) for a test item and the average that would be achieved by the reference laboratory if it undertook the same measurements on the same test item.

Calibration---------- It's a process of testing and adjusting an instrument or test system, to establish a correlation between the measurement response and the concentration of the substance that is being measured by the test procedure.

Coefficient of variation---------- A measure of variability or diversity associated with random error or imprecision. SD shows how much variation or dispersion there is from the mean (average or other Expected value) during repeated measures. A small SD indicates that data points tend to be very close to the mean, whereas a large SD indicates that the data points are spread over a wide range of values. SD is the square root of a dataset's variance.

Commutability------- Degree to which a material yields the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships for the same procedures applied to those types of material for which the procedures are intended.

Deviation index--------- A measure of how far a result differs from the mean value as multiples of the standard deviation.

External Quality Assurance (EQA)------ A programme which determines total testing performance by comparing a laboratory or clinic's test result (including interpretation of results) to a known standard or to an appropriate peer group mean generated from an inter-

	laboratory comparison in which multiple laboratories measure the same sample using the same test methods, reagents and controls.
Imprecision(random error) ----------	Lack of repeatability or reproducibility of the same result; represented by the standard deviation (in units of the test) or coefficient of variation**,** (in units of percent).
Inter-laboratory Comparability----------	The organisation, performance and evaluation of calibration/test results for the same or similar item by two or more laboratories in accordance with predetermined conditions
Inter-Individual Biologic Variation-----	Variation in the mean values when a quantity is measured in specimens from different individuals due to differences in homeostatic set point of the individuals
Inter-laboratory variability----------	It's the variability of test results from different laboratories using the same test method and analysing the same test material. It includes both within-laboratory and between-laboratory components of variance. Inter-laboratory coefficients of variation (CVs) are CVs calculated based on the total variance of results for a given method, endpoint,

and sample type.

Internal Quality Control--------- It involves the in-house procedures for continuous monitoring of operations and systematic day-to-day checking of the produced data to decide whether these are reliable enough to be released. The procedures primarily monitor the bias of data with the help of control samples and the precision by means of duplicate analyses of test samples and/or of control samples.

Intra-Individual Biologic Variation----- Variation in results when a quantity is measured in different specimens from the same individual obtained over a time span due to the imprecision of the measurement procedure (metrological variability) as well as to the rhythmic and random fluctuations of the quantity value around a virtual homeostatic set point.

Intra-laboratory Variability---------- Is the variability of test results from the same laboratory using the same test method and analysing the same test material. Within-laboratory coefficients of variation (CVs) are CVs calculated based on solely the within-laboratory component of variance.

Intra-Subject Variability ----------	Includes differences in test results due to both subject fluctuations and test performance fluctuations.
Matrix----------	The matrix of the specimen is defined as the totality of components of a material system except the analyte.
Matrix bias----------	The component of the observed difference due to non-commutability between a method/material combination.
Precision----------	Is the closeness of agreement between independent test results obtained under stipulated conditions.
Quality Control (QC)----------	Procedures which monitor analytical performance of instruments and detect analytical error. QC typically refers to use of quality control materials and analysis of resulting control data
Repeatability----------	Degree of consensus between successive measurements which have been done on the same sample with very similar conditions (same analyser, same user, same laboratory, same methods, same lot of reagents) in a very short time (e.g. same day). Often is symbolized as sr.

Reproducibility--------- Is the degree of consensus between successive measurements achieved on the same sample with different conditions (e.g. different analyzer, different user, different lot of reagents) in a long time. Can be either intra-laboratory or inter-laboratory, and is symbolized as sR.

Standard Deviation (SD)---------- A measurement of imprecision (random error), biologic variation, or other variability in a population; mathematically, CV is standard deviation divided by the mean and expressed as a percentage.

Trueness---------- Closeness of agreement between the average value obtained from a large set of test results and an accepted reference value

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LIST OF ABBREVIATIONS AND ACRONYMS

AMREF	African Medical Research Foundation
ANOVA	Analysis Of Variance
BA	Bland Altman
BGAs	Blood gas analysers
CBC	Complete blood count
CLIA	Clinical Laboratory Improvement Amendments
CPD	continuing professional development
CV	Coefficient of Variation
DI	Deviation Index
EA-REQAS	East African Regional External Quality Assessment Scheme
EDTA	Ethylene diamine tetra acetic acid
EQA	External Quality Assurance
EQAS	External Quality Assessment Schemes
ERC	Ethical Review Committee
HA	Haematology Analysers
Hb	Haemoglobin
HICN	Hemiglobincyanide
ICSH	The International Committee for Standardisation in Haematology
IQA	Internal Quality Assurance
IQR	Inter-quartile Range
ISO	International Organization for Standardization
KENAS	Kenya National Assurance Schemes

Km	Kilometre
KMLTTB	Kenya Medical Laboratory Technicians and Technologists Board
KNBTS	Kenya National Blood Transfusion Service
KNH	Kenyatta National Hospital
Lab	Laboratory
mL	Millilitre
NaCl	Sodium Chloride
NIQR	Normalized Inter-quartile Range
PBS	Peripheral blood smear
PCV	Packed Cell Volume
POC	Point- of- care
PT	Proficiency Testing
RBC	Red blood cells
RCC	Regional Co-ordinating Centre
SD	Standard Deviation
SOPs	Standard Operating Procedures
TLC	Total leukocyte count
tHb	Total Haemoglobin
UON	University of Nairobi
WBC	White blood cells
WHO	World Health Organisation

ABSTRACT

Accurate determination of haemoglobin (Hb) concentration is a common element in assessing the extent of anaemia and an important variable in directing transfusion therapy in patients. Laboratory measurements should be reproducible and consistent from day to day as well as between laboratories so that comparable results will be obtained when a given specimen is tested in different laboratories. Variability in Hb measurements is caused by many factors such as laboratory error, numerous physiologic, temporal and methodologic factors. Therefore this study was aimed at determining the interlaboratory variability of Hb measurements obtained from selected clinical laboratories across Kenya. A total of 188 public and 105 private laboratories selected from a total of 21 out of 47 Kenyan Counties received three specimens with low (A), normal (B) and high (C) Hb concentrations for analysis, after which their results were compared and evaluated for accuracy. Laboratory performance was assessed using the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) criteria. Coefficient of Variation (CV) was calculated as a measure of interlaboratory variability while the accuracy of the analysers was evaluated using one-way analysis of variance (ANOVA). A total of 67.98%, 64% and 50.6% laboratories gave accurate results for samples A, B and C respectively. The results generated by the Celltac, Humalyzer Junior, Medonic, Mindray, Colourimeter, Hemocontrol and Sysmex analysers were not significantly different ($P>0.05$) from the reference values. However, the Diaspect and Sahli analysers underestimated the Hb readings, while Hemocue, Urit and Mission overestimated the Hb readings ($P<0.05$). Interlaboratory variation of 33.3%, 25.1% and 29.4% for samples A, B and C was recorded irrespective of the analyser a laboratory used. The CV for the automated, semi-automated and manual methods was 7.08%, 7.04% and 34.26% respectively. The interlaboratory variation in Hb measurements resulted from variation in methodologies and types of analysers. Regular participation in External Quality Assessment Schemes (EQAS) is essential in order to achieve interlaboratory comparability of Hb results. Laboratories should embrace automation and gradually replace manual with automated methods, which are more accurate and reliable.

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Accurate determination of Hb concentration is a common element in assessing the extent of anaemia and an important variable in directing transfusion therapy in patients. This decision should be made based on accurate, reliable and timely laboratory tests. The detection of anaemia based on Hb concentration in venous or capillary blood is used to estimate the prevalence of anaemia in populations, allocate resources to programmes, and target intervention programmes to vulnerable groups; it is also used to screen individuals for participation in programmes and to evaluate response to interventions (Morris *et al.*, 1999). The Hb test is used to indirectly asses the oxygen carrying capacity of blood thus making it an important aid in establishing the presence of anaemia and treating anaemia. The measurement of blood Hb is one of the most common routine clinical laboratory tests. Hb determination may be requested as an individual test or as part of a complete blood count (CBC), which may be performed using either capillary or venous blood. The Hb test is precise, simple to perform, easily standardised and may be performed either manually or by using automated Hb analysers (Barbara, Anna & Norma, 2000).

The gold standard for assessing Hb concentration is the direct cyanmethaemoglobin method (Burger and Pierre-Louis, 2003) and the International Committee for Standardisation in Haematology (ICSH) recommends the Drabkin's method as the

standard method for determining the Hb concentration of whole blood (WHO, 2008). Lately, new continuous, non-invasive methods of measuring Hb have been introduced into the clinical environment whose accuracy may vary substantially from invasive Hb measurements. This variability in measurements is caused by many factors such as laboratory error, numerous physiologic, temporal and methodologic factors (Lauren, 2013).

Despite the variability of Hb measurements, clinicians rely on accurate Hb values to make decisions on transfusion and management of patients. Previous studies conducted in other parts of the world have shown variation of Hb estimation between laboratories (Blerk *et al.*, 2007; Bilto, 1999; Renu *et al*., 2007).

The World Health Organisation (WHO) EQA for haematology assesses the participating laboratories to correctly quantify the Hb level and number of white blood cells and platelets (World Health Organisation, 2007). Patient Safety Monitoring in International Laboratories (JHU-SMILE) remotely monitors approximately 165 international laboratories and clinics in 22 countries, 84 of which are in sub-saharan Africa. It is well known that laboratories in sub-saharan African countries face many challenges to provision of quality results (WHO, 2008; Frean *et al.*, 2012). A 40 month study of the EQA performance of the Sub-Saharan African JHU-SMILE laboratories reported that there was 0.6% failure rate for haemoglobin in that cohort of laboratories (Amukele *et al.*, 2012).

In East Africa, AMREF offers a number of programmes to address laboratory quality, including continuing professional development, competency assessments, blinded re-checking of test materials, regular structured support supervision and an

East African Regional External Quality Assessment Scheme (EA-REQAS). EA-REQAS was established in 2003 when the four Ministries of Health of East Africa (Kenya, Uganda, Mainland Tanzania, and Zanzibar) agreed to share laboratory standards and materials; develop standard documents targeting both clinicians and laboratory staff; establish national quality assurance bodies; form an EA-REQAS Committee to oversee activities; and appoint AMREF as the Regional Co-ordinating Centre. Seven surveys have been submitted since 2007, with nearly 400 participating facilities currently. Reference laboratories were identified to produce quality PT (Proficiency Testing) materials. There has been progressive improvement in performance by a few facilities participating in all surveys and national policy on laboratory methodologies has been influenced (3rd Regional Technical Meeting EA-REQAS, 2010)

1.2. Statement of the Problem

Although quality assurance programmes play an integral part in clinical laboratories of developed countries, these programmes have not been accorded the same degree of importance in the laboratories of developing countries. In Kenya in particular, only few laboratories have participated in such schemes implying that the Hb measurements of majority of clinical laboratories may not be accurate and comparable. In addition, the variability of the Hb measurements between clinical laboratories of Kenya has not been quantified. In order to address this problem, this study sought to asses the extent of inter-laboratory variations in Hb estimation in Kenyan clinical laboratories and to determine if the discrepancies are potentially large enough to affect decisions consequently impacting on patient treatment and

management. Further, the study sought to evaluate the accuracy of the most commonly used Hb analysers in Kenyan clinical laboratories.

1.3. Justification

Systematic error or bias may affect the measurement of a sample such that the result obtained is not always a perfect value but may be some distance from the true value (Kaplan, Pesce, & Kazmierczak, 2003). Use of different methodologies, reagents and assay conditions (e.g. temperature, reagent concentrations, detection methods, wash steps etc) are the reasons for the poor agreement between analysers. To quantify these differences, methods need to be validated using reference methodologies to ensure accuracy and comparability of results across laboratories (Fierdoz, 2012).

Laboratories must continuously strive to maintain and improve the quality of laboratory results, which is a never-ending process. Using statistical methods, laboratories can continually improve their performance and the reliability of patient results as well. Quality assurance in haematology is intended to ensure the reliability of the laboratory results. A quality assurance programme has two main aspects, namely, internal quality control (IQC) and external quality assessment (EQA) (Sah, Raj & Prakash, 1999). IQC and EQA play very crucial roles in ensuring reliability of analytical results (Westgard, 2008). EQAS of clinical laboratories ensures between-laboratory comparability of results as well as detecting bias (systematic errors) and overall review on the IQC programme (Rippey & Williamson, 1988; Savage 1989).

Poor selection of techniques, lack of essential equipment, lack of quality control materials and quality assurance systems, personnel issues and shortages of supplies

are some of the challenges to providing quality services faced by laboratories in developing countries (Carter *et al.,* 2002). Significant exchange of information in patient care, teaching and research is becoming more and more difficult due to the steadily increasing variation in methods and equipment (Munich, 1982). It is therefore important that interlaboratory variability of Hb measurements and reliability of equipment/analysers and methods used for Hb measurements in Kenyan clinical laboratories be assessed to enhance quality. Despite the existence of the EQA programmes in Kenya, however, laboratory participation is still very low and optional. Therefore, it is essential that studies such as this present one are frequently carried out to help in the improvement of interlaboratory comparison.

1.4. Research Questions

1. Can the laboratories participating in EQA of Hb measurements accurately differentiate normal, low and high Hb specimen?
2. Is the inter-laboratory variability of the Hb measurements in an external quality assessment quantifiable?
3. Can the variability of Hb measurements among the various Hb measurement methods/equipment be compared?

1.5. Hypothesis

1.5.1 Null Hypothesis

There is no difference in Hb measurements obtained from selected clinical laboratories in Kenya.

1.5.2 Alternative Hypothesis

There is difference in Hb measurements obtained from selected clinical laboratories in Kenya.

1.6. Objectives

1.6.1. General Objectives

To determine the inter-laboratory variability of Hb measurements obtained from selected clinical laboratories in Kenya.

1.6.2. Specific Objectives

1. To determine the ability of participating laboratories to accurately differentiate normal, low and high Hb specimens.
2. To quantify the inter-laboratory variability of the Hb measurements.
3. To compare the variability of Hb measurements among the various Hb measurement methods/equipment.

CHAPTER TWO

LITERATURE REVIEW

2.2. Variability of Hb measurements

Both accuracy (how close the measurement is to the actual value) and precision (how repeatable the measurement is) of "standard" laboratory measurements are subject to numerous methodologic factors that affect them (Lauren, 2013). Various methods of Hb determination have been used all through ((Barbara, Anna & Norma, 2000). In the 1950s numerous different methods were in use leading to variability of the results. This led to several attempts being made to standardise the measurement of the Hb concentration in human blood. The Haemiglobincyanide method was well accepted, soon came into general use, and performed satisfactorily for many years.

Later, with automation of laboratory methods in laboratory medicine, various methods came up. This led to the manual Haemiglobincyanide method being phased out as a routine method and became the reference method with which the current methods should agree (Zigilstra, 1997). Measurement of total haemoglobin (tHb) concentration by an automated laboratory analyser or point-of-care (POC) analyser, using a venous or capillary blood sample is the conventional method. ICSH has recommended the Drabkin's method as the standard method for determining the Hb concentration of whole blood (Chen *et al.,* 1992).

The clinical measurement of tHb has inherent variability. Inter-device variability is one cause of variability, for example, CO-Oximeter and POC devices that are commonly used to measure Hb have been shown to vary up to ±1.2 and ±1.3 g/dL,

respectively. Additionally, there are a variety of physiologic and methodologic factors that can significantly influence Hb levels in the body (Masimo, 2009).

2.1. Impact of Physiologic factors on haemoglobin measurements

A variety of factors influence Hb measurement and Hb levels in the body; these include both the laboratory devices and physiologic factors. Sources of Hb variation within the body include, the type of blood sample, sample site, time the sample is taken, and body position.

2.1.1. Type of Blood Sample

Laboratory devices are designed to allow sampling of either venous or arterial blood. Hb measurement may vary based on whether arterial or venous blood is used. arterial Hb measurements can be expected to be, on average, 0.7 – 1.0 g/dL less than the Hb measurements derived from venous blood (Mokken et al., 1996 & Yang ZW et al., 2001) The percentage of plasma concentration can vary from the arterial to venous blood based on a variety of physiologic factors, despite the total amount of circulating red blood cells and Hb remaining relatively constant whether in arterial or venous blood. The amount of plasma concentration can be higher in arterial blood, potentially leading to lower concentration of Hb (Masimo, 2009)

2.1.2. Sample Site

The site on the body from where blood is drawn can also affect Hb measurements. Large discrepancies were found between the values obtained from capillary blood samples from the left and right hands of the same women, with intrasubject standard deviation of 0.8 g/dL and correlation of 0.7 (Morris et al., 1999). The wide limits of

agreement indicate that two samples from different fingers of the same person could have Hb concentrations that differ by up to 2.0g/dL. Another study shows wide variation in the Hb concentration of capillary blood samples obtained from different fingers on the same individual at the same time. Intrapatient variability ranged as high as 7% (Bouton et al., 1994)

2.1.3. Time

Even in stable patients, Hb measurement can vary significantly over time. In a study of venous blood samples drawn from the same individuals on two different occasions, within person variances could vary as much as 2.6 g/dL in males and 2.3 g/dL in females (Looker AC, et al., 1990 & Burger et al., 2004). In another study, when Hb measurements were taken from the same individual on four (4) different days consecutively, intrasubject variability was 7.0% and the standard deviation was 0.8 g/dL ((Bouton et al., 1994)

2.1.4. Body Position

The position of the body before and during the blood draw also affects Hb measurements due to the normal composition of blood, interstitial fluid shifts, and elevations of protein and white blood cells. Body position has a significant effect on venous Hb measurements due to decreases in plasma volume on assuming an upright position. Heart rate and blood pressure are higher when standing vs. sitting, which induces the movement of intravascular fluid such as plasma into interstitial compartments. This causes Hct and Hb levels to rise (haemoconcentration) and plasma volume to decrease (Martin et al., 1997). Gore and colleagues showed a 6%

reduction on plasma volume with standing, which changed Hb up to 2 g/dL (Gore CJ, et al., 1992). Moving from seated to standing positions for 20 minutes may result in a change in Hb concentration by >1.0 g/dL (Daniel-Johnson et al., 2007). The converse is also true, indicating that if patients who are ambulatory change body position prior to the blood draw, they may require a period of equilibration.

2.2. Laboratory devices and their impact on variability of Hb measurements

Historically venous, arterial, or capillary blood samples have been used for Hb measurement; POC devices that use capillary blood samples. However, recently new continuous, non-invasive methods of measuring Hb have been introduced into the clinical environment whose accuracy can be variable compared to laboratory Hb measures.

As clinicians interpret laboratory measurements, they expect that the values would not change significantly if consecutive samples were measured repeatedly on the same laboratory device or on different laboratory devices (Lauren, 2013). According to the International Organization for Standardization (ISO), laboratory error is "failure of a planned action to be completed as intended, or use of a wrong plan to achieve an aim, occurring at any part of the laboratory cycle, from ordering, examinations to reporting results and appropriately interpreting and reacting to them" (Plebani & Lippi, 2010).

Care providers should take responsibility from the time of receipt of a request for a pathology test/investigation, to the time outcomes are communicated to the requester (The Royal College of Pathologists of Australasia, 2010). Therefore efforts to

reduce errors should consider the total testing procedure and quality improvement should be measurable in terms of improvement to patient safety and clinical care outcome (Plebani, 2010; Plebani, 2009). The chance of inappropriate care due to laboratory errors ranges from 6.4 % – 12 %, although up to 30 % of errors also resulted in patient discomfort, an escalation of costs and unwarranted additional testing (Lippi & Guidi, 2007). Laboratory error, physiologic, temporal and methodologic factors are the causes of the variability in reported Haemoglobin values (Lauren, 2013). Multiple variables, which include device calibration, sample handling, and other sources of variation specific to the technology, affect the accuracy of each of the different methodologies used for total Hb measurement (Lauren, Stephanie & Erin, 2011).

Both intra-device and inter-device variability affect total Hb measurements. Intra-device comparison is Hb variability from the same blood sample on the same device while inter-device comparison is Hb variability from the same sample on different devices (Gehring *et al*., 2007). Previous studies have shown that there is significant inter-device and intra-device variation in the Hb measurements; in 2007, five (5) different models of CO-Oximeters were used to evaluate both inter-device and intra-device variation in Hb measurements. When the same blood sample was analysed on 2 identical devices to test intra-device variability, the standard deviation between measurements ranged from 0.2 to 1.2 g/dL (Gehring *et al*., 2007). Both reference devices and test devices produce and/or contain inherent errors (Bland and Altman, 1986). The tHb measurements using a venous sample can vary as much as 0.9 g/dL

between different laboratory analysers across the normal tHb measurement range, using a reference calibrator (RNA Medical, 2011).

Despite the use of POC haematology analysers becoming more frequent in the last decade it has been shown that POC devices for Hb have reduced accuracy compared to laboratory devices. Device methods, size of the blood sample, and strong potential for confounding elements with capillary blood are factors that affect POC device accuracy. At normal Hb levels of 13-15 g/ dL, the Clinical Laboratory Improvement Amendments (CLIA) specification variance is approximately 1.0 g/dL while in the anaemic range of 10 g/dL, the target variance is 0.7g/ Dl (Masimo, 2009). Previous studies reveal a significantly larger difference in haemoglobin measurement between POC and laboratory devices; Hb measurement from capillary blood in POC devices varies between 0.5 – 2.3 g/dL from reference standards (Gomez-Simon *et al.*, 2007; Patel *et al.*, 2007; De Louw *et al.*, 2007; Argawal & Heinz, 2001).

2.3. Accuracy and precision of Hb measurements

Inter-laboratory imprecision as assessed by proficiency testing comprises components of intra-laboratory imprecision and, more importantly, inter-laboratory dissimilarities. Greater precision of techniques usually indicates a higher degree of system stability among sites. Performance specifications for dispersion of results and limits of acceptability are usually based on a percentage difference and /or absolute difference from the target value (Rej & Jenny, 1992).

The Clinical Laboratory Improvement Act of 1988 (CLIA, 1988) define fixed limit goals in absolute terms or multiples of standard deviations for a particular analyte. According to CLIA 88 proficiency testing criteria, acceptable analytical performance for haemoglobin is target ± 7% (CLIA, 1988).

The desirable specifications for within-subject biological variation, between-subject biological variation, Allowable Total Error, Imprecision, and Bias (inaccuracy), for Hb derived from Intra- and Inter-Individual Biologic Variation are 2.8%, 6.6%, 4.1%, 1.4% and 1.8% respectively (Ricos *et al.*, 1999).

Many studies that have been conducted in many parts of the world have quantified the imprecision, and accuracy (bias) of Hb measurements using the standard deviation, coefficient of variation and the deviation index. The EQAS in haematology at National Institute of Health, Islamabad, Pakistan (IEQAS) conducted a survey to assess individual values against consensus value (mean ±SD) and deviation index (DI) from the mean, whereas coefficients of variation (CV) were calculated for years 1996 to 2006. The results were expressed as percentage of accurate versus inaccurate results, DI and CV. The laboratory achieved 87.74% of values within acceptable limits for Hb, 72.03% for white blood count, 69.49% for platelet and 77.03% for reticulocyte estimation. These results were satisfactory, having DI values less than 3 for all four parameters. Results were varied among individual components of the survey but the overall DI values lay fairly well within the acceptable limits with a majority of the results having a DI value less than 3. Those results having DI values less than 3 were further classified into three categories; excellent (DI > -2.0 to < 1.0), satisfactory (DI 1.0 to < 2.0) and out of acceptable limits (DI < -2.0 or > 2.0). Overall, the laboratory achieved 64.22 % of

values with excellent results, 15.24% of results were within acceptable limits and graded as satisfactory whereas 20.52% of values showed a high deviation from the reference range. Mean DI values ± standard deviation and CV for Hb, were calculated for each year. DI + standard deviation (SD) for Hb ranged between -0.6±0.7 to 2.4±1.3.The study concluded that Participation in EQAS is extremely beneficial for the improvement of laboratory performance and quality of care (Birjees *et al.*, 2009).

2.4. Quality control in haemoglobinometry

Regular calibration of spectrophotometers and Hb analysers used for Hb assays, as specified by the manufacturer, must be done in order to achieve reliable Hb values. Daily run of appropriate control solutions or when patient samples are run, recording of control results and maintenance of records is a requirement. In addition each instrument used must have its own particular checks that must be performed (Barbara, Anna & Norma, 2000). Use of calibration specimens and high quality control Hb solutions; checking and calibration of equipment are crucial for proper determination of Hb in the blood. The results of Hb determination in control solutions obtained in autoanalysers and manually, are comparable (Pupkova *et al.*, 2002).

Since 1996 the Hb standard (lot number BK-5182-2, later renamed lot 19-1-B518A) has been in use till in 2008 when a new lot of the haemiglobincyanide (HiCN (Fe), HiCN) standard was (#19-1-B806) was released by the ICSH in conjunction with Eurotrol, B.V. The Haemoglobincyanide standard is used for the standardisation and calibration of whole blood Hb measurements on most haemoglobinometers and

automated blood cell counters. This new lot was produced following the same methodology previously specified by ICSH. Later both the WHO Expert Committee on Biological Standardisation (International Reference Reagent 98/708) and the European Community Bureau of Reference endorsed this lot as an international reference material for Hb (Davis & Jungerius, 2010).

2.5. External Quality Assessment

Currently, EQAS exist in the field of laboratory medicine in many countries. Most of these are intended to assist individual laboratories to continuously monitor their performance and to compare it with that of other laboratories, whereas others may be mainly intended for accreditation or licensing purposes. Additionally, EQAS may monitor the quality of the commercial analytical systems, reagents and test kits, and they help manufacturers to achieve a better harmonization of results from the different analytical techniques (Ricos *et al.*, 1996).

The two main aims of EQAS are to set both the target values and limits for acceptance.

Reference methods are used to assign the target values, but since only a few schemes follow these principles, target values are derived from the statics of each survey (Ricos *et al.*, 1996). Continual participation in EQA is an effective means for identifying and mitigating variables that influence the reliability of analytical assays for predictive markers, thereby assisting in technical validation and standardization. The concept of EQA for the national health laboratories network is useful, as it identifies problems in the comparability of laboratory results and initiates a process

towards solving these problems thus improving the quality of service at the level of each individual laboratory and the network level (Olafsdottir *et al.*, 1994).

The implementation of a quality assurance policy in a developing country requires a commitment from the government, the professional societies and the laboratory workers. It is important to recognize that a policy towards improving health care should include an external surveillance system for health laboratories. An EQA scheme will have the greatest impact when it is linked to a quality assurance programme, which also includes internal quality control as an equally important component (Deom *et al.*, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was a cross-sectional study using inter-laboratory survey to assess the extent of inter-laboratory variability of Hb assays in the clinical laboratories of Kenya. The study lasted a period of 6 months (Between February and July 2016).

3.2. Study site

This study covered all the different laboratory categories which included national reference laboratories, accredited laboratories, district & sub-district laboratories, health centre laboratories and private hospital laboratories (level A, B, C, D and E respectively). The study covered a total of 21 out of 47 counties in Kenya namely; Bomet, Embu, Isiolo, Kajiado, Kericho, Kiambu, Kirinyaga, Kisii, Kitui, Laikipia, Machakos, Makueni, Meru, Muranga, Nairobi, Nakuru, Narok, Nyandarua, Nyeri, Tharaka Nithi and Uasin Gishu. The main study site was the Central Laboratory, African Medical and Research Foundation, Kenya Located in Nairobi, from where the samples were prepared and distributed to all the participating laboratories.

3.3. Study Population

The study population consisted of both Public and private clinical laboratories. All laboratory categories, that is, national reference laboratories, accredited, district & sub-district, health centres and private hospital laboratories were considered. A total of 827 privately-owned laboratories, 2 national reference laboratories, 4 accredited

laboratories, 136 district hospitals, 130 sub-district and 1040 health centre laboratories formed the study population from which the sample size was calculated.

3.4. Sample size

The sample size was calculated, as shown below, for the different levels of laboratories using the population for each level. The number of laboratories countrywide in each laboratory category represents the population from which a representative sample was drawn. The following are the populations for the different laboratory groups as indicated in the master facility list and as reported by the KMLTTB (Kisabei, 2013, pers. comm).

Table 3-1: Categories of Clinical laboratories in Kenya

CATEGORY OF LABORATORY	NUMBER
National reference laboratories	2
Accredited laboratories	4
District hospital laboratories	136
Sub- district hospital laboratories	130
Health centre laboratories	1,040
Private laboratories (Includes mission hospital laboratories)	827

Using Cochran's sample size formula for continuous data (Cochran, 1977);

$$n_o = \frac{t)^2 * (s)^2}{(d)^2}$$

Where t = value for selected alpha level of 0.05(.025 in each tail) = 1.96

Where s = estimate of standard deviation in the population = 2.333

(Estimate of variance deviation for 14 point scale calculated by using 14 [inclusive range of scale] divided by 6 [number of standard deviations that include almost all (approximately 98%) of the possible values in the range]).

Where d = acceptable margin of error for mean being estimated = 0.42 (Number of points on primary scale * acceptable margin of error; points on primary scale = 14; acceptable margin of error = 0.03 [error researcher is willing to accept]). Therefore d = 14 * 0.03 = 0.42.

3.4.1. District hospital laboratories and Sub- district hospital laboratories

Population = 136+130 =266 laboratories

$$n_o = \frac{t)^2 * (s)^2}{(d)^2} = \frac{(1.96)^2 (2.333)^2}{14 *0.03)^2}$$

=118

However, since this sample size exceeds 5% of the population (266 *.05=13.3), Cochran's (1977) correction formula was used to calculate the final sample size. These calculations are as follows:

$$n_i = \frac{n_o\ (118)}{(1 + no / Population)} = \frac{118}{(1 + 118/266)} = 81$$

Where n0 = required return sample size according to Cochran's formula= 118.

Where n1 = required return sample size because sample > 5% of population.

Sample size = 81(40 district and 41 sub-district hospital laboratories)

3.4.2. Private laboratories

Population ----------827

$$n_o = \frac{t)^2 * (s)^2}{(d)^2} = \frac{(1.96)^2 (2.333)^2}{14 * 0.03)^2}$$

=118

Correction formula:

$$n = \frac{n_o (118)}{(1 + no / Population)} = \frac{118}{(1 + 118/827)} = 103$$

3.4.3. Health centre laboratories

Population----------1,040

$$n_o = \frac{t)^2 * (s)^2}{(d)^2} = \frac{(1.96)^2 (2.333)^2}{14 * 0.03)^2}$$

=118

Correction formula:

$$n = \frac{n_o (118)}{(1 + no / Population)} = \frac{118}{(1 + 118/1040)} = 105$$

3.4.4. Accredited laboratories

Population----------4

Sample size----------3

3.4.5. National referral laboratories

Population ----------2

Sample size----------1

The total sample size selected was … 81+103+105+3+1 =293 laboratories

Table 3-2: Summary of the sampled laboratories for EQA

CATEGORY OF LABORATORY	NUMBER
National reference laboratories	1
Accredited laboratories	3
District hospital laboratories	40
Sub- district hospital laboratories	41
Health centre laboratories	105
Private laboratories	103
TOTAL	**293**

3.5. Sampling Method

Cluster random sampling of the laboratories from the 21 counties was used. To arrive at the laboratory, a two-stage cluster sampling method was used. The counties represented the clusters from which the laboratories were randomly selected. From each county, a minimum of 7 public and 5 privately owned laboratories were sampled. All laboratory categories were represented in all the counties except for national reference and accredited laboratories which were sampled from the counties they are located. Laboratories were selected from the rural, semi urban and urban settings.

3.5.1. Inclusion Criteria

Both public and privately-owned laboratories that perform Hb measurements as one of their routine laboratory tests and gave free informed consent for participation were included in the study. All methods of Hb determination being used in the laboratories were applicable.

3.5.2. Exclusion criteria

Private laboratories, which are not registered by KMLTTB (Kenya Medical Laboratory Technicians and Technologists Board), and all laboratories that are further than 315 kilometres (km) from the Central Laboratory, AMREF, Kenya were excluded from the study because of logistical challenges.

3.6. Laboratory procedures

3.6.1. Preparation of EQA samples

EQA samples with low, normal and high Hb values (samples A, B and C) respectively were prepared at the Central Laboratory AMREF, Nairobi (EAREQAS, operating under African Medical and Research Foundation, Kenya).

3.6.2. Preparation of haemolysate

Three EQA samples with low, normal and high Hb values (samples A, B and C) respectively were prepared as follows:-

One pint (450 ml) of blood in a blood bag which had tested negative for Human immunodeficiency virus, hepatitis B virus surface antigen, syphilis and hepatitis C virus antibodies was obtained from the Kenya National Blood Transfusion Service (KNBTS). Using a centrifuge tube, 40 ml of the blood was centrifuged to separate plasma and Buffy coat aseptically. To the red blood cells deposit, 2-3 fold volume of physiological saline (9 g/L Sodium Chloride)(NaCl) was added, mixed well and centrifuged at 2000g for 5 minutes. The supernatant and any remaining buffy coat were discarded completely. This saline wash was repeated 2 times to ensure complete removal of plasma, white cells and platelets. To the washed cells, half its

volume of carbon tetrachloride (neat), 99%, was added and the mixture shaken vigorously in a mechanical shaker (Vibrofix VF1, Janke and Kunkel Model) at 2500g/minute for one hour. The mixture was then stored at 4^{o}C overnight thus forming a semi-solid interface of lipid/cell debris between carbon tetrachloride and lysate. The mixture was centrifuged at 2500g for 20 minutes and the upper lysate layer was carefully pipetted out into a clean Winchester bottle. Sterility and stability of the haemolysate was maintained by the addition of preservatives and broad-spectrum antibiotics as follows: To each 70 ml of lysate, 30 ml of glycerol was added followed by addition of 25-50 mg of penicillin and 25-50 mg of gentamicin per 500 mL of material. To make haemolysate with lower Hb concentration, an appropriate volume of 30 % (v/v) glycerol in 9 g/L NaCl was added to the stock and mixed well using a roller for one hour. While stirring continuously, 1 mL aliquots were dispensed aseptically into 2 mL sterile vials, capped, sealed and labelled appropriately. The samples were assigned unique codes A, B and C for sample with low, normal and high Hb concentration respectively. The samples were preserved at $2\text{-}8^{0}$ C in the refrigerator awaiting dispatch. An elaborate procedure of how the samples were prepared is annexed (Appendix I).

3.6.3. Assigning value of Hb concentration to the EQA samples (target values)

The Hb concentration for the three samples was assigned using the reference method i.e. by use of a standard haematology analyser (Sysmex XS-800i, Sysmex Corporation, Kobe, Japan) as the gold standard. These were used as the target/reference values against which the results of the participating laboratories

were compared. The target/reference values for the three samples were 6.2 g/dl, 13.6 g/dl and 18.1 g/dl for sample A, B and C respectively.

3.6.4. Sample handling and storage

In order to minimise pre-analytical errors before and after sending materials to the laboratories strict measures regarding sample aliquoting and sample handling were taken. When preparing aliquots only one vial was handled at a time to avoid exposing the samples to room temperature for more than 10 minutes. Samples were checked for any leakage, spillage or contamination and for correct labelling with well sticking labels. They were then stored according to temperature requirements until testing can be performed, that is at 2-8^{0}C.

3.6.5. Sample packaging and transportation

The sample package for each participating laboratory contained three samples labelled sample A, sample B and sample C. The samples were placed in leak proof 2 ml plastic vials which were properly labelled with the unique code numbers. The quality of the samples was maintained during transport by use of icepacks so as to maintain temperatures of 2-8^{0}C. Samples were also secured during transport so that there is no leakage, spillage or contamination. During transport to the laboratories a triple packaging system was used which consisted of a leak-proof primary receptacle, a leak-proof secondary packaging with sufficient absorbent material and an outer packaging of adequate strength (Appendix II). An efficient and reliable means of transport was used.

In order to ensure that all the necessary requirements were followed by the participating laboratories, the samples were accompanied by a complete set of instructions with respect to storage, handling and deadline for analysis. The instruction sheet contained information about the number of samples, type of samples and details of how the samples are labelled, how to mix samples before testing and a deadline for analysing the samples. Laboratories were informed to perform checks for the samples before testing such as the storage conditions, code numbers, breakage/ leakage, sign of contamination and the temperature condition at which the samples were received (Appendix III).

3.6.6. Sample processing

The laboratories were instructed to process the samples within two days after delivery using their current analytical procedures, to process the samples in the same way as routine samples and record the results in the worksheet provided. Samples were analysed in duplicate by performing two assays with a difference of not more than six hours between the assays.

Alongside the study, each laboratory also received a questionnaire so as to collect data on analytical aspects related to Hb determination. Data on analytic aspects of Hb determination included, methods of analysis used, reference ranges used by each laboratory, control materials and reagents used for analysis, use of Standard Operating Procedures and previous participation in EQAS. Demographic data included the code (assigned by Principal Investigator), location and mailing address of the participating laboratories. The laboratories were also required to state clearly, by filling the questionnaire, the date the samples were received, date of analysis and

the type of equipment/method used. The results were collected from the participating laboratories within one week after dispatch of the samples.

3.7. Data Management and Analysis

Data was entered in Ms excel worksheet, coded and edited using consistency checks, checks for duplicate entries and range checks. Data was analysed using XLSTAT statistical software (XLSTAT Version 2013.3.03). For inter-laboratory variability, coefficient of variation (CV) was used. The higher the CV the greater the dispersion or spread in values of the variable, whereas when the CV is lower, the residuals relative to the predicted value are smaller. The variation was also assessed using analysis of variance (ANOVA). Significant differences ($P<0.001$) between means were assessed by ANOVA. For evaluation of performance of participating laboratories, acceptable performance criteria given by the CLIA' 88 was used. Accuracy of results was analysed by calculating the difference (expressed as the bias) between the Hb concentration provided by the participating laboratories and target values. We tested for the effect of the Hb concentration on the proportion of the laboratories that performed well based on CLIA'88 criterion using Chi-Square analysis. Data presentation was done using tables and graphs.

3.8. Ethical Considerations

Approval to carry out the study was sought from the Jomo Kenyatta University of Agriculture and Technology as well as Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (Ref: KNH – ERC/A/1) (Appendix VIII). Consent was obtained from the laboratory managers of the participating laboratories

prior to enrolment in the study. Confidentiality was maintained by coding the participating laboratories rather than using their names. The questionnaires and worksheets were kept under lock and key so that only the Principal Investigator could access them.

CHAPTER FOUR

RESULTS

A total of two hundred and ninety two laboratories responded. Twenty seven different analysers were used across all the laboratories (Table 4-3).

Table 4-3: The Different Analyzers and the Number of Laboratories Using Each Analyser Type

Analyzer	Method of Operation	Number of Hb Measurements	Number of Laboratories
ABX Micros	Automated	6	1
ACT Diff Beckman Coulter	Automated	18	3
BTS 305	Manual	12	2
Celltac	Automated	174	29
Cera Check	Manual	18	3
Colourimeter	Semi- Automated	78	13
Coulter Counter	Automated	24	4
Diaspect	Manual	306	51
Drew	Automated	6	1
Easy Mate	Manual	12	2
Sahli	Manual	144	24
Hb Meter	Manual	12	2
Hemocontrol	Manual	264	44
Hemocue	Manual	384	64
Hichroma	Automated	6	1
Humalyzer Junior	Semi- Automated	30	5
Hybrid	Automated	12	2
Kyrot	Automated	6	1
Medonic	Automated	30	5
Mindray	Automated	60	10
Mission	Manual	36	6
Pentra ES 60	Automated	6	1
RMS	Automated	6	1
Stat	Manual	12	2

Sysmex	Automated	48	8
Urit	Manual	30	5
Erma	Automated	12	2

4.1. The performance of participating laboratories in differentiating, low, normal and high Hb measurements

Based on the CLIA'88 acceptable performance criteria for haemoglobin, which is, target ± 7%, a total of 61% of laboratories had acceptable performance across all measurements. The analyses shown in table 4-4 revealed that laboratory performance using CLIA'88 criteria declined with increase in the concentration of the target Hb value: 68%, 64% and 51% of laboratories passing for the sample with low (6.2g/dl), normal (13.6g/dl) and high (18.1g/dl) Hb values respectively. These differences were statistically significant ($p < 0.001$) for the three Hb test concentrations for both reading 1 and reading 2 (Table 4-4).

Table 4-4: The Number and Percentage of Laboratories that Performed Well (Passed) or Failed according to CLIA' 88 Test Performance Criteria

Measurement	Reading 1			Reading 2			Average		
	Passed	Failed	**Percent success**	Passed	Failed	**Percent success**	Passed	Failed	**Percent success**
Low (A)	198	94	**67.81**	199	93	**68.15**	397	187	**67.98**
Normal (B)	191	101	**65.41**	183	109	**62.67**	374	210	**64.04**
High (C)	148	144	**50.68**	148	144	**50.68**	296	288	**50.68**
Chi square			21.16			19.50			40.30
P value			**P<0.001**			**P<0.001**			**P<0.001**

4.2. The inter-laboratory variability of the Hb measurements

The overall inter-laboratory CV was 33.3% for sample A, 25.1% for sample B and 29.4% for sample C irrespective of the analyser a laboratory used (fig 4-1). When inter-laboratory CV was calculated across laboratories using the same analyser, the CV reduced to 5.1 % (Hemocontrol) to 41 % (Urit) for sample A, 2.2% (Celltac) to 35% (Diaspect) for sample B and 3.4 % (Medonic) to 42.6% (Diaspect) for sample C (Table 4-5).

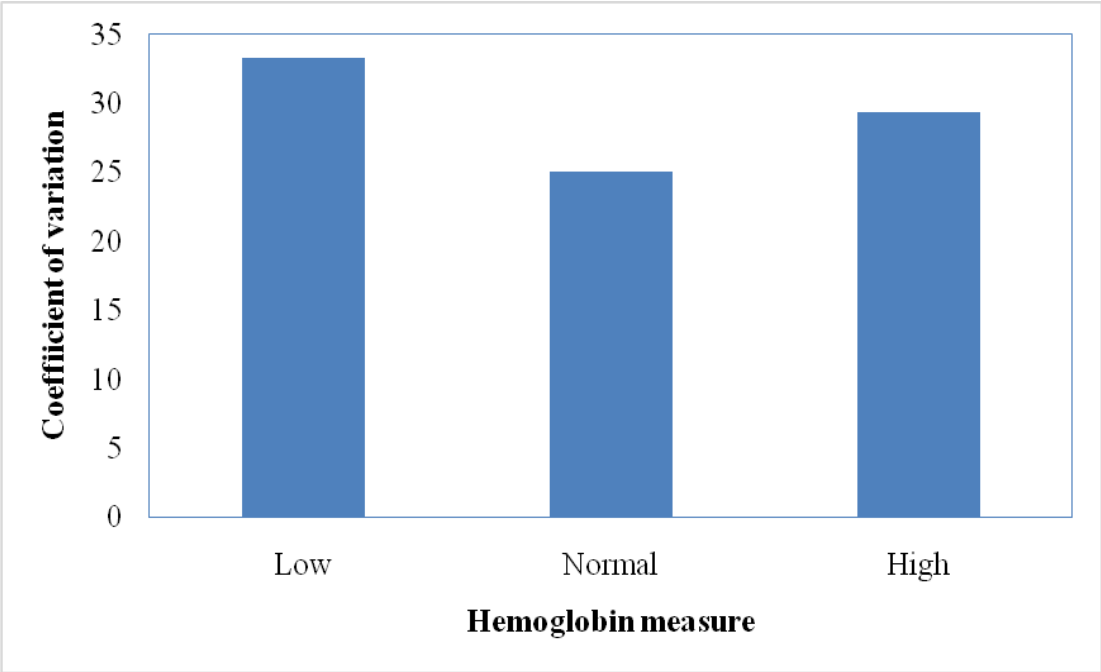

Figure 4-1: Coefficient of variation as a measure of inter-laboratory variation for the three Hb test concentrations

Table 4-5: The Inter-Laboratory Variability of Hb Measurements for Each Type of Hb Analyser

		Mean ± 1 Standard Deviation			CV (%)		
Hb Analyzer	**n**	**A**	**B**	**C**	**A**	**B**	**C**
Celltac	29	6.43±1.09	13.83±0.30	18.7±0.97	16.9	2.2	5.2
Colorimeter	13	6.64±1.18	13.57±1.79	17.39±1.72	17.8	13.2	9.9
Diaspect	51	4.06±0.68	9.61±3.36	9.37±3.99	16.8	35.0	42.6
Hemocontrol	44	6.25±0.32	13.96±0.51	18.86±0.84	5.1	3.6	4.4
Hemocue	64	8.37±2.37	16.16±3.09	21.14±4.10	28.3	19.1	19.4
Humalyzer Junior	5	6.10±0.43	13.00±1.07	17.64±0.84	7.1	8.3	4.8
Medonic	5	6.84±1.56	14.22±1.85	18.54±0.63	22.9	13.0	3.4
Mindray	10	5.99±0.6	13.33±0.48	18.35±0.78	10.1	3.6	4.3
Mission	6	11.25±2.62	16.52±1.49	19.67±1.14	23.3	9.0	5.8
Sahli	24	5.80±1.83	10.44±2.55	13.2±2.89	31.6	24.4	21.9
Sysmex	8	6.23±0.52	13.43±0.73	18.25±1.07	8.3	5.5	5.8
Urit	5	8.44±3.47	17.36±4.98	21.76±3.07	41.1	28.7	14.1
Average	**269**	**6.465+2.12**	**13.39+4.95**	**17.08+3.26**	**32.85**	**24.38**	**28.98**

4.3. Comparison of the variability of Hb measurements among the various Hb measurement methods/equipment

4.3.1. Variation of Hb measurements due to analysers

Table 4-6 shows the accuracy of Hb analysers in estimating low, normal and high Hb measurements. The values obtained by the different analysers and methods were compared to the mean of the reference values. The mean Hb measurements from the various analysers were significantly different from the mean of the reference values ($F_{(27)} = 17.382$, $P<0.001$,). In spite of this, seventy eight percent (n=21/27) of the Hb analysers produced mean Hb values that were not significantly different from the mean of the reference values and only 22% (n=6 of 27) had mean values deviating significantly from that expected mean of the reference values. Most of the common analysers produced estimates of Hb that were consistently different or similar to the reference values for sample A, B and C (Tables 4-7, 4-8 & 4-9). The exception was Sahli and Mission. Sahli gave Hb estimates that were similar to the reference value for sample A but gave results that were significantly different than the reference values for sample B and C. Mission gave estimates that were significantly different from sample A and B reference values but not for sample C reference value.

Table 4-6: The Accuracy of Analysers in estimating Hb values across all levels of measurement in comparison to the expected or mean of all reference values (6.2, 13.6, and 18.1) combined (i.e. 12.633)

Analyzer	N	Mean Hb	Value	Standard. Error	T. Statistic	P value
Intercept	162	12.63	12.633	0.403	31.353	< 0.001
ABX Micros	6	11.63	1.000	2.132	-0.469	0.639
Beckman coulterAct Diff	18	12.92	0.283	1.274	0.222	0.824
BTS 305	12	10.53	2.100	1.534	-1.369	0.171
Celltac	174	12.99	0.356	0.560	0.636	0.525
Cera Check	18	10.89	1.739	1.274	-1.365	0.173
Colourimeter	78	12.6	-0.035	0.707	-0.049	0.961
Coulter Counter	24	12.52	-0.117	1.122	-0.104	0.917
Diaspect	**306**	**7.68**	**-4.951**	**0.498**	**-9.935**	**< 0.001**
Drew	6	13.45	0.817	2.132	0.383	0.702
Easy mate	12	15.57	2.933	1.534	1.912	0.056
Hb Meter	**12**	**16.76**	**4.125**	**1.534**	**2.688**	**0.007**
Hemocontrol	264	13.05	0.416	0.512	0.812	0.417
Hemocue	**384**	**15.23**	**2.593**	**0.480**	**5.398**	**< 0.001**
Hichroma	6	12.75	0.117	2.132	0.055	0.956
Humalyzer Junior	30	12.09	-0.540	1.019	-0.530	0.596
Hybrid	12	11.84	-0.792	1.534	-0.516	0.606
Kyrot	6	13.5	0.867	2.132	0.406	0.684
Medonic	30	13.13	0.497	1.019	0.487	0.626
Mindray	60	12.54	-0.090	0.775	-0.116	0.908
Mission	**36**	**15.73**	**3.092**	**0.945**	**3.272**	**0.001**
Pentra Es 60	6	12.77	0.133	2.132	0.063	0.950

RMS	6	12.45	-0.183	2.132	-0.086	0.931
Sahli	**144**	**9.81**	**-2.826**	**0.587**	**-4.812**	**< 0.001**
Stat	12	14.96	2.325	1.534	1.515	0.130
Sysmex	48	12.64	0.010	0.843	0.012	0.990
Urit	30	**15.85**	**3.217**	**1.019**	**3.156**	**0.002**
Erma	12	12.51	-0.125	1.534	-0.081	0.935

Table 4-7: The accuracy of the most commonly used analysers in Kenya in estimating low Hb value (6.2 g/dl)

Analyzer	Method of operation	N	Mean	Value	Standard. error	T statistic	P value
Intercept				6.200	0.284	21.823	< 0.001
Celltac	A	29	6.43	0.234	0.395	0.594	0.553
Colourimeter	SA	13	6.64	0.438	0.498	0.880	0.380
Diaspect	M	51	4.06	-2.137	0.351	-6.083	**< 0.001**
Hemocontrol	M	44	6.25	0.048	0.361	0.132	0.895
Hemocue	**M**	**64**	**8.37**	**2.167**	**0.339**	**6.397**	**< 0.001**
Humalyzer Junior	SA	5	6.1	-0.100	0.719	-0.139	0.889
Medonic	A	5	6.84	0.640	0.719	0.890	0.374
Mindray	A	10	5.99	-0.210	0.546	-0.384	0.701
Mission	**M**	**6**	**11.25**	**5.050**	**0.666**	**7.579**	**< 0.001**
Sahli	M	24	5.8	-0.396	0.414	-0.956	0.340
Sysmex	A	8	6.23	0.025	0.594	0.042	0.966
Urit	**M**	**5**	**8.44**	**2.240**	**0.719**	**3.117**	**0.002**

KEY:

A – Automated

SA – Semi Automated

M - Manual

Table 4-8: The accuracy of the most commonly used analysers in Kenya in estimating normal Hb value (13.6 g/dl)

Analyzer	Method of operation	N	Mean	Value	Standard error	T statistic	P value
Intercept				13.6	0.447	30.418	< 0.0001
Celltac	A	29	13.83	0.231	0.621	0.372	0.71
Colourimeter	SA	13	13.57	-0.031	0.784	-0.039	0.969
Diaspect	M	51	9.61	-3.988	0.553	-7.213	< 0.0001
Hemocontrol	M	44	13.96	0.357	0.568	0.628	0.53
Hemocue	**M**	64	16.16	2.556	0.533	4.795	< 0.0001
Humalyzer	SA	5	13	-0.6	1.131	-0.53	0.596
Medonic	A	5	14.22	0.62	1.131	0.548	0.584
Mindray	A	10	13.33	-0.27	0.86	-0.314	0.754
Mission	**M**	6	16.52	2.917	1.049	2.782	0.006
Sahli	M	24	10.44	-3.158	0.652	-4.846	< 0.0001
Sysmex	A	8	13.43	-0.175	0.935	-0.187	0.852
Urit	**M**	4	17.36	3.76	1.131	3.324	0.001

KEY:

A – Automated

SA – Semi Automated

M - Manual

Table 4-9: The accuracy of the most commonly used analysers in Kenya in estimating high Hb value (18.1 g/dl)

Analyser	Method of operation	N	Mean	Value	Standard error	T statistic	P value
Intercept				18.100	0.540	33.498	< 0.001
Celltac	A	29	18.7	0.600	0.751	0.799	0.425
Colourimeter	SA	13	17.39	-0.708	0.948	-0.747	0.456
Diaspect	M	51	9.37	**-8.733**	**0.668**	**-13.069**	**< 0.001**
Hemocontrol	M	44	18.86	0.759	0.686	1.106	0.270
Hemocue	**M**	64	21.14	**3.038**	**0.644**	**4.714**	**< 0.001**
Humalyzer Junior	SA	5	17.64	-0.460	1.367	-0.337	0.737
Medonic	A	5	18.54	0.440	1.367	0.322	0.748
Mindray	A	10	18.35	0.250	1.039	0.241	0.810
Mission	**M**	6	19.67	1.567	1.267	1.236	0.217
Sahli	M	24	13.2	-4.900	0.788	-6.221	< 0.001
Sysmex	A	8	18.25	0.150	1.130	0.133	0.895
Urit	M	5	22.63	4.525	1.504	3.008	0.003

KEY:

A – Automated

SA – Semi Automated

M - Manual

4.3.2. Variation of Hb measurements due to methods

A total of 74.32%, (n=217) of the laboratories used analysers/machines that employed a manual method, 24% used an automated methods (n=70) and 1.7%, (n=5) used semi-automated methods (fig 4-2). ANOVA comparisons revealed that the mean Hb across the three methods of analyser/machine operation were not significantly different from the mean of the reference values ($F_{(3)}$ =1.333, P=0.262, mean of reference value =12.633). When we calculated the CV using the mean of reference values, the CV for the automated and semi automated methods were similar but was large for analysers using the manual method (CV for automated analyzers =7.08%, CV for semi automated analyzers = 7.04% and CV for manual analyzers =34.26% (fig 4-3).

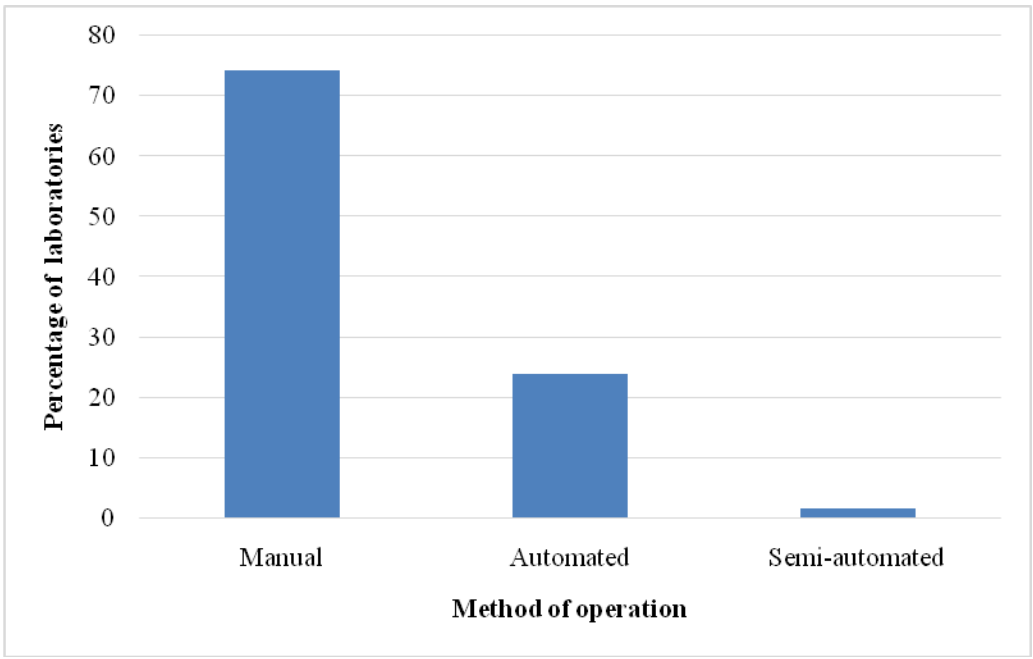

Figure 4-2: Percentage number of laboratories as per each method of equipment /analyser operation

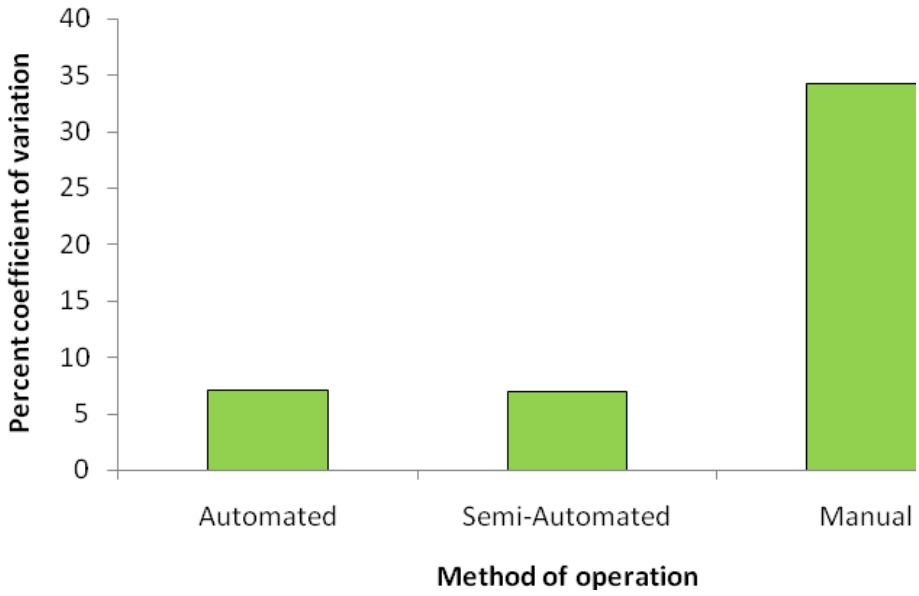

Figure 4-3: Coefficient of variation around the standard reference as a measure of reliability of the different methods of Hb analyser operation

4.4. Resuts for the questionnaire

Important analytical data regarding the practice of internal quality control in the laboratories was as shown in table 4-10.

Table 4-10: The resuts for the questionnaire

S/No	Question	Number Of Laboratories	Percentage
1.	Used SOPs during testing	200	68.5 %
2.	Previous participation in EQA	44	15%
3.	Used quality control materials	131	45%
4.	Reported the correct reference values for Hb	254	87%
5.	Used manual methods	217	74.3%
6.	Used automated methods	70	24%
7.	Used semi-automated methods	5	1.7%

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

As demonstrated in this study, 61% of laboratories met the CLIA'88 criterion for samples A, B and C (Table 4-4). The ability of a laboratory to accurately determine the Hb values of a sample depended on the type of analyser/method used and the application and adherence to quality control practices by the laboratory. The results in this study have demonstrated variation of Hb results when laboratories analyse the same sample. Such results will have potential implications in the classification of anaemic patients. For instance, assuming sample A, B and C was representative of an anaemic subject, a healthy normal subject and a subject with higher Hb concentration respectively. A total of 1.7 % (5 out of 292), 19.8 % (58 out of 292) and 37.3 % (109 out of 292) laboratories respectively, would have misclassified these cases.

The present study demonstrates inter-laboratory variation in Hb measurements for the three Hb samples, which was slightly higher at low Hb concentration. Patients usually obtain health care services in different clinical settings and therefore may have their Hb levels measured in different laboratories using different methods that may not be comparable. In this study the range of Hb results obtained for each analyser/method varied widely. Confusion may arise when laboratories give different results for the same sample. For instance, in the present study there was a considerable high overall interlaboratory variation. However, when the analysers

were grouped according to the type of analyser, the variation of Hb results reduced to a great extent. This suggests that both method/analyser bias and laboratory-specific bias are the cause of the overall variability of interlaboratory Hb results.

Unfortunately, the interlaboratory variation may have consequences for clinical practice depending on the case at hand. The results obtained in the present study are similar to those found in a survey of Belgian hospitals to assess the reliability of Hb measurements which reported interlaboratory variation ranged from 0.6 % (Roche) to 6.7 % (IL) for sample 1 and from 2.0 % (Radiometer) to 4.5 % (IL) for sample 2 (Blerk *et al.,* 2007). Also, a study carried out to assess the analytical quality of Jordanian haematology laboratories in the routine haematological parameters reported that there was considerable inter-laboratory variation in laboratories using manual methods (Bilto, 1999).

In this study, some analysers gave results that are comparable to the reference values while as others overestimated and others underestimated the values. An overall negative bias was observed for Hb results obtained using Diaspect and Sahli. Analytic bias can directly affect patient classification and clinical decision making (Klee, 1995). In addition, underestimation and overestimation of Hb concentrations could lead to unnecessary clinical interventions in patients. Some analysers gave results that are comparable to the reference values while as others overestimated and others underestimated the values.

The underestimation of Hb values by both Diaspect and Sahli appears to increase with an increase in Hb concentration. These results supports findings from other

studies, which reported that Sahli method underestimated Hb values. According to Patil, Thakare and Patil, (2013) Sahli underestimates the Hb in capillary blood by 0.62gm/dl and 1.12g/dl in venous blood. Kapil *et al.,* (2002) compared HemoCue® analyser with Sahli's method and they found that Sahli's method underestimates Hb by 1.06 g/dl compared to HemoCue® analyser. Barduagni *et al.,* (2003) compared the performance of the Sahli and the colour scale methods in diagnosing anaemia in school children and found that Sahli method gave low mean Hb level (11.4g/dl) and had low specificity (39.0%), thus giving a high rate of false positive results. The considerable variability in the Hb values obtained with the Sahli could be due to its inbuilt errors, subjective visual colour comparison, inaccuracy in pipetting of blood, fading of comparator after prolonged use and poor sensitivity and reliability. Contrary to the results presented in this study, a study conducted to evaluate the utility of the Diaspect as a point of care analyser reported that the Hb values obtained with Diaspect system compared well with the reference analyser (Robertson, Lewis, & Osei-Bimpong 2011). It is evident from the findings of this study that both Diaspect and Sahli methods may classify healthy individuals as anaemic.

On the other hand, Hemocue, Urit and Mission were consistently overestimating the Hb values when compared to the reference. Overestimation of Hb values by Hemocue and Urit appears to increase with an increase in Hb concentration with an exception of Mission where the overestimation decreases with an increase in Hb concentration. Hemocue, Urit, Mission showed a positive bias in that order of increasing bias.

The reliability, precision and accuracy of hemocue is controversial. The results of this study showed that hemocue overestimated Hb values by more than 2g/dl from the reference and this overestimate appears to increase with increase in the actual Hb value. The present study findings agree with Mohanram, Ramana-Rao & Sastry, (2002) who observed that hemocue method overestimated Hb by more than 2g/dl. Prakash *et al.,* (1999), in their study on utility of hemocue in estimation of Hb against standard blood cell counter reported that hemocue produced consistently higher values of Hb than the blood cell counter and suggested that a constant correction factor of 0.5g/dl should be subtracted from Hb estimates of hemocue. Bhaskaram *et al.,* (2003) also found that hemocue overestimated Hb values by 10% - 15% when they compared it to cyanmethaemoglobin method in their study on validation of Hb estimation using hemocue and this overestimate appeared to increase with increase in the actual Hb value. However other studies such as those conducted by Sari *et al.,* (2001*)* to estimate the prevalence of anaemia among Indonesian mothers, Von Schenck, Falkensson and Lundergerg, (1986) in evaluation of hemocue and Rechner *et al.,* (2002) on evaluation of hemocue compared with the coulter STKS have found hemocue to be reliable and accurate and have supported its use. Yet, a study conducted in Mexico reported that hemocue underestimated Hb values (Neufeld et al., 2002). However, due to these contradicting findings on the accuracy and reliability of hemocue, it is suggested that further studies need to be done to validate the hemocue haemoglobinometer.

The findings in this study about Urit agree well with those of Jitthai (2012) who reported that Urit gave significantly higher Hb values than the automated blood

analyser. Generally, the variation of Hb values was slightly higher at low and high Hb concentrations and least at normal Hb concentration suggesting that majority of the laboratories may be using calibrators for the normal Hb concentration only while calibrating the analysers and fail to include calibrators for low and high Hb concentrations. The results presented in the current study demonstrate that the problem of inter-laboratory variability even between those laboratories that use the same brand of analyser is still big suggesting that there are inconsistent calibrations of instruments at the laboratory level. It is apparent that the variation of Hb measurements varies across the type of analyser used and the methods.

One possible explanation for deviant results from the different laboratories could have been due to "matrix effects". Processed blood haemolysate is prepared in a matrix that is less complete than patient's specimens and may have properties that differ from pure blood. For example, addition of stabilizers or other additives that are used in the preparation of processed blood samples are known to alter the properties of the specimen (Rej, Jenny and Bretaudire, 1984., Fasce *et al.*, 1973, Breutaudire *et al.*, 1981, Ulda, 1984, Fraser & Peake, 1980, Rej & Drake, 1991). Such anomalies are considered to be "matrix effects" (Clark, Kricka & Whitehead, 1981). Although matrix effects might explain some of the evident method- specific biases reported in this study, and which is expected to be more or less constant across all analyzers (Miller & Kaufman, 1993; Power, 1995). The widely variant results obtained with Sahli, Diaspect, Hemocue, Mission and Urit are less likely to be caused by matrix effects but may rather reflect serious calibration problems with these instruments in the field. However, other causes of bias that can result in false

test results include instrument calibration errors, reagent lot differences, inaccurate dilutions, personal performance error and other factors (Klee, 1999; Jenny & Jackson, 2000; Miller, Levinson & Elin, 2003.

The present study has demonstrated that there is considerable high variation of Hb values when using manual methods when compared with the automated methods. All the analysers that produced Hb values that deviated significantly from the expected mean values employed the manual method of operation. Also as is evident in this study, the manual methods showed higher CVs than the automated methods indicating that the automated methods generally have higher precision than the manual methods. These results agree with those of a previous study conducted by Fink *et al.,* (1997) where manual methods produced greater CVs than the automated methods. Also, as reported by Bilto (1999), in his study carried out to assess the analytical quality of Jordanian haematology laboratories, the cell counter methods showed better inter-laboratory agreement than the manual methods. Inherent errors in the manual methods of Hb value estimation on a sample and errors caused by the observer would be the causes of this great variation seen in the manual methods. Use of calibration specimens and high quality control Hb solutions; checking and calibration of equipment are crucial for proper determination of Hb in the blood (Pupkova *et al.,* 2002). These apparent differences in Hb results obtained by different laboratories using different analysers/methods (inter-device) and in different laboratories using the same analysers/methods (intra- device) can be addressed by harmonization of procedures for Hb measurement in all the laboratories.

The results obtained from the questionnaire indicate that very few (15%) laboratories participate in External Quality Assessment Schemes/PT programmes (Table 4-10). A possible explanation to this may be due to lack of funds since EQA is expensive, lack of awareness by some of the laboratories and lack of commitment by the laboratory staff and management as well. Regular participation in EQA assists individual laboratories to continuously monitor their performance and to compare it with that of other laboratories. Also, it is an effective means for identifying problems that cause interlaboratory variation of laboratory results and initiates a process towards solving these problems thus improving the quality of service at the level of each individual laboratory. Lack of participation in EQA is one of the factors among others, which cause interlaboratory variation of laboratory results.

Different laboratories used different reagents and control materials depending on the method of analysis/analysers used in their laboratory. In order to achieve standardisation and interlaboratory comparability of Hb results, manufacturers should consider coming up with a single common type of calibration material that can be used for all analytical methods and analysers. A total of 55% of laboratories reported not using quality control/reference materials when performing Hb test. This could be due to the fact that quality control materials are expensive and are not readily available to most laboratories. A close observation of the results indicate that even those laboratories that use quality control materials do not use all the three levels but only the normal Hb concentration.

A good number (31.5%) of the laboratories did not use SOPs when performing Hb tests, however, most laboratories reported not experiencing any challenges in the analysis of the samples. These poor quality control practices further increase the problem of interlaboratory variation of Hb measurements. Recent recommendations from the European External Quality Assessment–Organizers Working Groups A and B (Libeer *et al.*, 1996; Thienpont *et al.*, 1995), the European Union directive for in vitro diagnostic medical devices (Directive 98/79/EC 1998) the International Organization for Standardization (ISO 17511:2003, 2007) and the Clinical and Laboratory Standards Institute (CLSI Document X5-R, 2006) have recognized the objective of harmonized results and the importance of using commutable reference materials for method calibration traceability and for proficiency testing (PT) programs. To ensure standardisation of Hb measurements throughout the world, The International Council for Standardization in Haematology (ICSH) in conjunction with Eurotrol, B.V. (Ede, NL; http://www.eurotrol.com) released a new lot of the haemiglobincyanide (HiCN(Fe), HiCN) standard in 2008 for use in the standardization and calibration of whole blood Hb measurements on most haemoglobinometers and automated blood cell counters (Davis & Jungerius, 2010). However this reference standard is not readily available in the developing countries laboratories. There was a slight variation in the reference ranges being used in the laboratories which can have impacts on patient classification.

5.2. Conclusions

Laboratories participating in EQAs of Hb measurements are able to accurately differentiate low and normal Hb specimens and to a lesser extent, high Hb specimen.

Inter-laboratory variability of Hb measurements in an external quality assessment is quantifiable. Inter-laboratory variation of Hb results for samples with low, normal and high Hb concentrations was 33.3%, 25.1% and 29.4% respectively.

The results have shown that Celltac, Humalyzer Junior, Medonic, Mindray, Colourimeter, Hemocontrol and Sysmex produce accurate and reliable Hb results than Diaspect, Sahli, Hemocue, Urit and Mission.

The manual methods produced higher CVs than the automated methods thus indicating that manual methods generally have lower precision.

5.3. Recommendations

- Clinical laboratories need to regularly participate in continuous EQAS in order to achieve interlaboratory comparability of Hb results.
- There is need for an oversight body and policies to be established by the government that will oversee the implementation of method comparison studies in both private and public hospital laboratories in order to ensure interlaboratory and inter-method comparison of analytical results for Hb.
- This study has established the need for validation and standardisation of all analysers, methods and procedures of Hb measurement being used in the Kenyan clinical laboratories in order to achieve interlaboratory comparability.
- Laboratories should embrace automation and gradually replace manual methods with automated methods of Hb measurement which are more accurate and reliable.

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APPENDICES

Appendix I: Procedure for Haemolysate Preparation

STANDARD OPERATING PROCEDURE FOR PREPARATION OF HAEMOLYSATE SAMPLE

1. Centrifuge anticoagulated blood from a blood bank, with negative serology for the human immunodeficiency (HIV) hepatitis B (HBsAg), hepatitis C (HCV) virus in screw capped bottles of appropriate size. Aseptically separate the plasma from the white layer (layer of leukocytes or buffy coat).
2. Add to each red cell deposit an excess of physiological saline 9g/L (NaCl), mix well and centrifuge. Discard the supernatant and any remaining buffy coat.
3. Repeat saline wash two times with sterile physiological saline solution to the complete elimination of the plasma, leukocytes, and platelets, each time removing the top layer of the package of erythrocytes in each wash.
4. Add to the washed cells an equal volume of water and 0.5 volumes of carbon tetrachloride (CCl_3). Cap the containers and shake them vigorously in a mechanical Shaker for 1 hour. Refrigerate overnight to allow the lipids/cell debris to form a semi-solid interface between carbon tetrachloride and lysate.
5. On the following day, centrifuge at 2 500 g for 20 minutes; transfer the upper layers into universal containers and centrifuge at about 3000g for one hour. Collect the upper 95% and pool into a clean tube.
6. To each 70 mL of lysate, add 30 mL of glycerol. Then add antibiotics (25-50 mg of penicillin) and 25-50 mg of gentamicin per 500 mL of material.

This material is stored at 4 ° c until use for short periods or frozen at -20 for longer period until required for dispensing.

7. If a lower concentration is required, add an appropriate volume of 30% (v/v) glycerol in a solution of 9 g/l of sodium chloride (NaCl) to the stock. Mix well.
8. With continuous stirring distribute the mixture aseptically into sterile containers. Cover and seal.
9. Assign the haemoglobin value. Preserved at 4 ° C, the product should keep the value that has been assigned for several months.

Appendix II: Standard Operating Procedure for Triple Packaging System

STANDARD OPERATING PROCEDURE ON TRIPLE PACKAGING SYSTEM

The triple packaging system consists of three layers: the primary receptacle, the secondary packaging and the outer packaging. This packaging system is meant to withstand leakage of contents, shocks, temperature, pressure changes and other conditions that can occur during ordinary handling in transportation.

1) Put the sample in a primary receptacle (tubes/vials) and ensure that all the tubes/vials that are being sent are tightly closed to avoid any leakage of the sample. The primary receptacle containing the specimen must be watertight, leak proof and appropriately labelled as to content.
2) Wrap every tube/vial in enough absorbent material like paper towels or cotton wool to absorb all fluid in case of breakage or leakage.
3) Place the samples in a second watertight, leak proof packaging such as a zip lock bag so as to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in a single secondary packaging.
4) Get a plastic container and place the samples in the zip lock bag into it and close it.
5) Place four frozen ice packs from the -20 degrees Celsius compartment of the refrigerator, on the sides of a small cool box ensuring that the secondary container is in the middle of the ice packs.
6) Place absorbent material on top and if necessary between the ice packs.

7) Place the case investigation forms or appropriate laboratory request forms in a plastic bag to keep from becoming contaminated or destroyed by the wet ice packs.

   Note: An itemized list of package contents must be included between the outer and secondary container.

8) The cool box is then sealed and addressed to the receiver.
9) Label the box with biohazard symbol and with upward arrows

Note: The outer package must be of adequate strength for its capacity, mass, and intended use. It must be must be rigid and have one side that is at least 100 mm X 100 mm, in order for required markings and labels to fit.

Appendix III: Instructions for the Participating Laboratories

INSTRUCTIONS FOR HAEMOGLOBIN TEST SAMPLE HANDLING FOR EQAS IN HB MEASUREMENT

Inter-laboratory variability of haemoglobin measurements obtained from selected clinical laboratories in Kenya

Introduction

This is a set of instructions for the above study that should be followed by every participating laboratory so as to ensure proper sample analysis is done.

You are kindly asked to read keenly the instructions below before performing any analysis of the samples. Feel free to seek any clarification from the Principle Investigator.

Instructions to be followed before and during sample analysis:

1) Always keep the samples refrigerated at 2 – 6°c when not in use. Do not freeze the samples.
2) Perform sample analysis within two days after the delivery of the samples to your laboratory.
3) Verify that the samples are in good condition and well labeled before the analysis. There are three samples labeled sample A, Sample B & Sample C. Each vial contains exactly 1ml of sample.
4) Let the sample attain room temperature before carrying out the test.
5) Mix the samples well before the test by gently inverting the vial about 3 – 4 times.

6) Make sure that your machine/ equipment is in good working condition before the analysis.
7) Perform a single haemoglobin test of each of the samples in the morning hours using the analytical method that is currently being used in your laboratory. Record the results in the worksheet provided.
8) Perform the second haemoglobin test of each of the samples in the afternoon hours. Record the results in the worksheet provided.

   NOTE: The time difference between the morning and afternoon readings should not exceed 6 hours.
9) Lastly fill the questionnaire provided.
10) The results and the questionnaire will be collected from the laboratory within one week of sample delivery.

Appendix IV: Questionnaire

QUESTIONNAIRE FOR INTER-LABORATORY VARIABILITY OF HAEMOGLOBIN MEASUREMENTS STUDY

I am Esther Wangui Mandania, a master's student at Jomo Kenyatta University of Agriculture and Technology, faculty of COHES, Department of Medical Laboratory Sciences. I am conducting research on interlaboratory variability of haemoglobin measurements obtained from selected clinical laboratories in Kenya.

This is a qualitative questionnaire that is intended to provide the researcher with important information regarding haemoglobin measurement in the clinical laboratories of Kenya in order to assess the inter-laboratory variability of haemoglobin measurements.

Kindly provide the researcher with the following information;

1. Details for the laboratory:

   Code assigned to the laboratory (by PI) ----------

   Mobile Phone No: ----------

   Physical Address: P.O BOX----------

   Email Address----------

2. Which date and time were the samples received in your laboratory?

   Date----------Time----------

3. In which condition were the samples received by your laboratory?

   □ Intact (No damage/breakage)

   □ Damaged and leaking

   □ Labelled

   □ Unlabeled

4. What volume of each EQA sample did you receive?

   □Less than 0.5mL

   □ 0.5mL

   □ More than 0.5mL

5. Which equipment and method of haemoglobin estimation was used to measure haemoglobin of the EQA samples in your laboratory? Method?----------

   Equipment? ----------

6. Please specify the reference levels for haemoglobin used in your laboratory for the following;

   - Adult women----------
   - Adult men----------
   - Children----------

7. Which reagents did your laboratory use for haemoglobin estimation for the samples? ----------

   ----

   What challenges and difficulties did your laboratory experience in the analysis of the samples?----------

   ----

   State the SOP that your laboratory used during samples analysis? ----------

   ----

8. Please state the control materials that were used during samples analysis -----

   ---

   ---

9. Which EQA scheme for haemoglobin has your laboratory participated in before? ----------

   ---

Appendix V: Worksheet

Worksheet for inter-laboratory variability of Hb measurements study

Code of laboratory:		
Date & time of analysis:		
Results (g/dL)		
Type of EQA Sample	**Reading 1**	**Reading 2**
EQA Sample A		
EQA Sample B		
EQA Sample C		

Appendix VI: Ethical Clearance

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/1 Link:www.uonbi.ac.ke/activities/KNHUoN 6th January 2014

Mandania Esther Wangui
Reg.NO.TM300/1160-2012
Dept. of Human Pathology and Laboratory Medicine
JKUAT

KNH/UON-ERC P.O.Box 20723-00202NRB KENYATTA NATIONAL HOSPITAL APPROVED 06 JAN 2014

Dear Esther

RESEARCH PROPOSAL: LABORATORY VARIABILITY OF HAEMOGLOBIN MEASUREMENTS OBTAINED FROM SELECTED CLINICAL LABORATORIES IN KENYA (P458/08/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 6th January 2014 to 5th January 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N.Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
AD/Health Information, KNH
Supervisors: Juliette R. Ongus, Sarah N. Kaggia, Fatmah Abdallah

Appendix VII: Informed Consent Form for Laboratory Management

INFORMED CONSENT FORM

Inter-laboratory variability of haemoglobin measurements obtained from selected clinical laboratories in Kenya

Introduction

This informed consent form is for the laboratory in-charges, supervisors or managers of clinical laboratories that i am inviting to participate in research on haemoglobin measurement. The title of the research is "**Inter-laboratory variability of haemoglobin measurements obtained from selected clinical laboratories in Kenya"**

Principal investigator:

Name: Esther Wangui Mandania

Study Authority: Jomo Kenyatta University of Agriculture and Technology

Faculty: College of Health Sciences

Department: Medical Laboratory Sciences

Address: P.O Box 2028, Thika

Phone No: 0727906351

E-mail: emandania@yahoo.com

Authorizing body: KNH/UON-ERC

This Informed Consent Form has two parts:

- **Information sheet (to share information about research with you)**
- **Certificate of consent (for signatures if you agree to take part)**

You will be given a copy of the full informed consent form

PART I: Information Sheet

I am Esther Wangui Mandania, a master's student at Jomo Kenyatta University of Agriculture and Technology, faculty of COHES, Department of Medical Laboratory Sciences.

I am doing research on variability of haemoglobin measurements between clinical laboratories in Kenya. I am going to give you information and invite you to participate in this research. Before you decide to participate in this research, you can talk to anyone you feel comfortable with about the research. Also ask any questions that you may have about the research. You do not have to decide today whether or not you will participate in the research, sufficient time will be given to you to make this decision.

If there is anything or any word that you do not understand, please ask me to stop as we go through the information and i will take time to explain.

Purpose of the research

Comparability of haemoglobin test results is difficult due to variability of haemoglobin measurements between laboratories. Laboratories should strive to produce reliable results that are consistent between laboratories so that comparable results for the same test are obtained in all the laboratories. The reason i am doing this research is to find out if laboratories can give the same results for the same test and to determine the variability of haemoglobin measurements between clinical laboratories in Kenya. The information obtained from the research will help to identify areas of improvement in haemoglobin measurements in the laboratories.

Procedures

- This research will involve analysis of three EQA samples only (A, B and C) which will be analysed the same day and filling the results of analysis in a worksheet.
- If your laboratory agrees to participate in this research you will receive three EQA samples labelled A, B and C accompanied by a questionnaire and a worksheet.
- I will request you to analyse the samples within two days in the routine way as patient samples are analysed, record the results in the worksheet provided and fill the questionnaire.
- You will then send the results and the questionnaire to the principal investigator or they will be picked from the laboratory whichever you prefer.

Duration

Your expected time commitment for this study will not be more than four days in total. However the investigator will take about 6 months to complete the whole research study.

Risks

There are no any risks for your participation in this study.

Benefits

If you (your Laboratory) participates in this research, you will receive a summary of your(laboratory's) performance which will include the results from the laboratory, the expected values (targets) and the potential causes of errors and remedial actions(for laboratories that will not be in a position to give the target values). This will provide an opportunity to the laboratory to identify the areas of improvement in the

measurement of haemoglobin as well as in general areas of your laboratory, which the laboratory can work on to improve the quality of the results.

It is hoped that your participation will help the researcher determine and learn more about variability of haemoglobin measurements between clinical laboratories in Kenya, how they impact on patient management and how inter-laboratory comparability of results can be achieved.

Confidentiality

The information that will be collected from this research project will be kept confidential. The results from your laboratory and all information about the laboratory will not be disclosed to any other laboratory or party. The results from your laboratory and any information about your laboratory will have a number on it instead of the name of the laboratory. Only the researcher will know what your laboratory number is. During dissemination of the research findings, this information will only be reported as group data with no identifying information. All data, including questionnaires and worksheets for the study will be kept in a secure location under lock and key and no one will have access to them except the researcher. After the research is complete, all the raw data that is, the questionnaires and worksheets will be destroyed by shredding followed by burning.

Compensation

You will not be given any compensation to take part in this research.

Voluntary Participation

Your Participation in this research study is entirely voluntary. It is your choice to decide whether to participate or not. Whether you choose to participate or not, this will not affect the relationship you have with the researcher. You may change your

mind later and stop participating even if you agreed earlier. Also you have the right to withdraw at anytime or refuse to participate entirely in the research study without any consequences. If you do decide to take part in this study, you will be asked to sign the consent form.

Dissemination of findings

Research findings will be disseminated through publications, conferences and through other scientific reports however, no identifying information will be used so as to maintain confidentiality and privacy. Also you will receive a summarised report of your laboratory's performance.

Who to contact

If you have any questions regarding this study, you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Principal Investigator:

Name: Esther Wangui Mandania

P.O BOX 2028, Thika

Mobile telephone: 0727-906351 or 0789-641355

E- Mail address: emandania@yahoo.com.

Supervisor:

Dr. Juliette R. Ongus

Jomo Kenyattta University of Agriculture and Technology

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KNH/UON-ERC Secretariat:

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Post address: P O BOX 20723-00202, Nairobi, Kenya

Physical Address: School Of Pharmacy, UON behind KNH Dental clinic

E-mail: knhuonerc@gmail.com

Website: www.ounbi.ac.ke

Link: www.uonbi.ac.ke/activities/KNHUoN

This proposal has been reviewed and approved by the KNH/UON-ERC. If you wish to find out more, contact the KNH/UON-ERC secretariat using the contact provided above.

PART II: Certificate of Consent

I have read the foregoing information and understood, i have had the opportunity to ask questions about it and any questions that i have asked have been answered to my satisfaction. I understand that my participation is voluntary and that i am free to withdraw at any time without giving any reason and without cost. I have received a copy of this consent form. I voluntarily agree to participate in this study.

Laboratory Code: ----------

Name of laboratory in-charge/supervisor/manager: ----------

Signature: ----------Date: ----------

Name of PI: ---------- ID No: ----------

Signature: ---------- Date: ----------