

**PREVALENCE OF TRANSFUSION TRANSMITTED
MALARIA AMONG BLOOD DONORS AND EVALUATION
OF CARESTART™ AND QUANTITATIVE BUFFY COAT
METHODOLOGIES AT KENYATTA NATIONAL HOSPITAL,
KENYA**

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Transfusion Science)**

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**Prevalence of Transfusion Transmitted Malaria Among Blood Donors and
Evaluation of Carestart™ and Quantitative Buffy Coat Methodologies at
Kenyatta National Hospital, Kenya**

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**A thesis submitted in partial fulfilment for the Degree of Master of Science
in Medical Laboratory Sciences (Haematology and Blood Transfusion
Science) of Jomo Kenyatta University of
Agriculture and Technology**

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DECLARATION

This thesis is my original work and has not been presented in any institution leading to the award of a degree or any other award.

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DEDICATION

To our son Moffat Muuo Kioko, for his curiosity and thirst for knowledge at tender age which drove him to go on hunger strike enthusiastically demanding to start schooling before age at Happyland Preparatory School, Kenya. You were motivated to write your own books, may you live to read and write big!

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AO	Acridine Orange
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra Acetic Acid
FN	False Negative
FP	False Positive
HBsAg	Hepatitis B surface Antigen
HCV	Human Cytomegalo Virus
HIV	Human Immunodeficiency Virus
HIV	Human Immunodeficiency Virus
MRDT	Malaria Rapid Diagnostic Test
MRDTs	Malaria Rapid Detection Tests
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
QBC	Quantitative Buffy Coat
RBCs	Red Blood Cells

RBCs	Red Blood Cells
RDT	Rapid Diagnostic Test
TN	True Negative
TP	True Positive
TTIs	Transfusion-Transmitted Infections
TTM	Transfusion-Transmitted Malaria
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Blood products	Any therapeutic substance prepared from human blood. This includes whole blood components and plasma derivatives.
Endemicity	Is a measure of disease prevalence in a particular region.
Haemolyzed	It is the bursting of red blood cells releasing heme, a substance in the blood that binds oxygen.
Malaria	An infectious disease caused by protozoan parasites from the Plasmodium family that can be transmitted by the bite of the Anopheles mosquito or by a contaminated needle or transfusion.
Mortality	The number of deaths in a given area or period, or from a particular cause.
Prevalence	Is the proportion of people infected at a given point in time.
Quantitative Buffy Coat	A method of diagnosing malarial parasites based on micro-centrifugation, fluorescence, and density gradient of infected red blood cells.
Rapid diagnostic Techniques	Are instrument-free tests that provide results within 20 min and can be used by community health workers.
Syndromic diagnosis	Method to diagnose malaria is syndromic diagnosis, in which the diagnosis is made on the basis of clinical history, signs and symptoms.

Transfusion	An act of transferring donated blood, blood products, or other fluid into the circulatory system of a person.
Voluntary blood donor	This is a person who voluntarily donates blood and its products majorly for therapeutic use.

ABSTRACT

Malaria is a disease of high economic importance that can be transmitted through blood transfusion in medical facilities. Blood and its products are not screened for TTM in transfusion setups in sub-Saharan Africa. The objectives of this study were to determine the prevalence of malaria parasite among voluntary blood donors, the performance characteristics and the level of agreement of two malaria screening techniques. The study applied a cross-sectional descriptive study design. The study involved recruitment of 155 voluntary blood donors. The blood samples were subjected to three malaria screening techniques which included microscopy, quantitative buffy coat and rapid diagnostic test. The study revealed that the prevalence of malaria infection among voluntary donors by microscopy was 3.2%, Quantitative buffy coat was 3.9% and rapid diagnostic test was 5.2%. The sensitivities of microscopy, rapid diagnostic test and quantitative buffy coat were 100%, 80% and 100% while the specificities of the three techniques were 100%, 97% and 99% respectively. The positive predictive values for microscopy, rapid diagnostic test and quantitative buffy coat were 100%, 97% and 83% and the negative predictive values were 100%, 99% and 100%. The kappa value for RDT was 0.63 and the kappa value for QBC was 0.90. The study revealed that Quantitative Buffy coat technique results compared favourably with microscopy because it picked almost all positives picked by the gold standard while Rapid Diagnostic Test yielded higher prevalence values above the other techniques because it picked more false positive values. The study revealed that quantitative buffy coat technique had higher sensitivity and specificity in detection of TTM than rapid diagnostic test. The study also established that there was substantial agreement between the gold standard and the RDT technique (0.63) and an almost perfect agreement between the gold standard and the QBC technique (0.90) therefore QBC was a viable screening technique for malaria parasites.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Malaria is a devastating mosquito borne illness that is predominantly prevalent in the tropical and subtropical regions of the world. Malaria is mainly transmitted through the bite of an infected female *Anopheles* mosquito. Transmission can also occur through transfusion of infected blood and its products. In malaria-endemic areas, epidemiological studies have reported a prevalence of malaria among potential blood donors to range between 1% and > 50% (Epidi, Nwani, & Ugorji, 2008). It has been estimated that nearly half of the world's population is at risk of contracting malaria with Africa being the most malaria risky area (Karunamoorthi, 2012). The risk of plasmodium transmission through blood transfusion is accounted for by persistence of malaria parasites in the blood.

Malaria is one of the world's deadliest diseases affecting people particularly in tropical and sub-tropical regions of the world., with 300 to 500 million cases and 2 to 3 million deaths per year (Ekwunife, Ozumba, Eneanya, & Nwaorgu, 2011). A recent study demonstrated a relatively high likelihood of TTM via transfusion in sub-Saharan African countries, illustrated by a median prevalence of malaria and determined by the evaluation of thick smears of 10.2% (range 0.7 % in Kenya to 55% in Nigeria) in donor blood samples (Owusu-Ofori, Betson, Parry, Stothard, & Bates, 2013).The disease imposes serious effects on the blood, destroying red blood cells and interfering with the hemoglobin. It affects the health as well as the wealth of individuals, ultimately the nations at large. This is often regarded as both a disease of poverty and a cause of poverty (Karunamoorthi & Bekele, 2009).

Malaria is caused by the intracellular protozoan parasite of the genus *Plasmodium* that invades and multiplies in the liver and red blood cells (RBCs) during its lifecycle in man (Noubouossie, Tagny, Same-Ekobo, & Mbanya, 2012). Malaria parasites are principally transmitted from an infected person to healthy human beings through infective bites of female *Anopheles* mosquitoes (Karunamoorthi, Sabesan, Jegajeevanram, & Vijayalakshmi, 2013). It can also be spread through blood transfusion from an infected donor. Transfusion transmitted malaria imposes a serious threat to human kind in the impoverished malaria endemic countries, and it may lead to serious problems because infection with *Plasmodium falciparum* may cause rapid fatality (Hassanpour et al., 2011). Malaria remains a complex and overwhelming health problem facing humanity. Over centuries, malaria remains not only as a major public health issue but also inflicts a serious negative socio-economic implication in resource poor settings (Karunamoorthi, Deboch, & Tafere, 2010; Karunamoorthi et al., 2013).

Some transfusion guidelines suggest that transfusion recipients should be given systematic anti-malarial prophylaxis (Adewuyi, 2001). As a result, presumptive anti-malarial treatment with inexpensive chloroquine was given to blood recipients to prevent TTM for many years. However, the spread of chloroquine resistance across Africa has led to such a strategy becoming redundant and ineffective. Alternatives to chloroquine, such as artemisinin and artemisinin-based combination therapy (ACT) are considerably more expensive, weakening the applicability and usefulness of anti-malarial prophylaxis among resource poor people. Persistent existence of counterfeit antimalarial has torn the society by imposing severe threat in all spheres of clinical and public health domains. Kenya being an endemic region, transfusion transmitted malaria (TTM) may result with relatively higher mortality, particularly among the vulnerable sections of the society like pregnant women, infants, splenectomized and immune compromised patients as a result of tardy diagnosis and treatment. Infected blood transfusion

directly releases a large number of malarial parasites in the recipients' bloodstream, who are already debilitated attributable to ill health (Dover & Schultz, 1971; Guiguemide et al., 1992; Mungai, Tegmeier, Chamberland, & Parise, 2001).

Worldwide, four malaria species: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* are responsible for transfusion induced malaria, as they are adapt to survive well in the stored blood even in frozen state (Talib & Khuana, 1996). *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax* species are the most frequently associated ones with the transfusion incidences (Kitchen, Barbara, & Hewitt, 2005; Mungai *et al.*, 2001). Blood products like RBCs, platelets and plasma are commonly transfused to treat various ailments and maladies (Shimosaka, 2007). Besides, it is also helpful to correct severe anemia, deficiency of plasma clotting factors thrombocytopenia, immune deficiency status and hypoalbuminemia (Kitchen & Elnageh, 1997; Talib & Khuana, 1996).

In many cases, the treatment of hematology or oncology disease is intensive and often causes severe leucopenia, thrombocytopenia, and anemia (Shimosaka, 2007). Strikingly, now and then, these blood components might be infected if the donors carry any pathogens such as protozoa, viruses, bacteria, syphilis, spirochete and nematode. Besides, sometimes, it may result in acute or delayed complications and carry the intrinsic risk of TTIs including HIV, hepatitis B and C, syphilis, and malaria too (Sawke, Sawke, & Chawla, 2010). WHO (WHO, 2012) recommends that all blood donations should be screened for malaria where appropriate and possible, and that there should be quality assured testing for transfusion-transmitted infections (TTIs). These recommendations have significant resource implications and have not been widely implemented by transfusion services.

Indeed, there are reasons for the difficulty in screening blood for malaria. Furthermore, it is important to note that there is an inherent risk of potential hazard in each and every phase of the blood transfusion chain and a failure at any of these stages can have serious negative implications in terms of recipients' health condition (WHO, 2010). However, apparently these complications and intrinsic risks can be substantially minimized by espousing appropriate safe blood management practices (Sawke *et al.*, 2010). Modern transfusion medicine plays a vital role to save and boost millions of lives every year from various killer infectious diseases and disorders.

However, infected or contaminated blood could lead to severe complications and ultimately death. It has a serious negative implication in the emerging economies, mainly in the malaria prone endemic settings in Africa (Abdullah & Karunamoorthi, 2005). It is purely as a result of inadequate resources and skilled personnel in order to collect, process, preserve, and administer the safe transfusion. Therefore, this scrutiny is an attempt to shed light on the existing issues of blood safety in malaria endemic countries and emerging opportunities or strategies and their own limitations for mitigating risk of blood borne pathogens, particularly malarial parasites.

It is believed that this communication could pave the way for policymakers, public health experts, and healthcare providers to design and implement strict guidelines and protocols for the safe blood transfusion practices. It could eventually save health as well as wealth of millions of needy people in the impoverished malaria endemic countries in the very near future. The World Health Organization advocates that blood donation should be replacement and voluntary and non-remunerated. Commercially remunerated donors tend to come from the poorest sectors of society, are more likely to live in densely populated, malaria infested, poor sanitary environments, more likely to be poor in health, under nourished, more

likely to give blood more often than is recommended and are more likely to transmit transfusion transmissible infections (Okocha *et al.*, 2005).

1.2 Statement of the Problem

Malaria as a transfusion transmitted infection causes morbidity and mortality in Kenya. Blood donations for transfusion have not been screened for malaria parasites. Instead; antimalarial drugs may sometimes be administered to recipients after blood transfusion without confirmation of malaria infection and the risks of post transfusion malaria infections are real. This is contrary to WHO recommendations which require laboratory screening of malaria parasite before initiation of treatment. This has not been implemented effectively here in Kenya. In addition, lack of an ideal malaria screening tool may have contributed to the lack of compliance.

Administration of antimalarial prophylactic drugs blindly may be wasteful and may trigger allergic reactions, facilitate the development of drug resistance and retard patient recovery through various mechanisms including over load of the liver. Establishing the proportion of transfused blood which is positive for malaria parasites is important but challenging. Furthermore, two malaria screening techniques were evaluated for their suitability in routine screening of blood collected for transfusion procedures.

1.3 Justification

Transfusion of blood that is positive for plasmodium parasites carries the risk of post transfusion malaria infection. Such infections may contribute substantially to cases of morbidity and mortality in Kenya. This study was aimed to mainstream the screening of blood

donations for malaria. This study was done to ensure that blood used in transfusion is safe to administer to patients and thus minimizes the hazards associated with the current practice: side effects of antimalarial treatment, drug resistance, wastage and the spread of the disease.

The study aimed in evaluating and made recommendations of the most suitable tools to use for malaria parasite screening of blood for transfusion. This study was also made to determine the prevalence of malaria infection amongst blood donations. The information generated from this study will inform practice and policy makers towards enhancing the quality of blood used in transfusions.

1.4 Research Questions

This study sought to provide answers to the following questions: -

1. What was the prevalence of malaria parasites among blood donors using rapid diagnostic and quantitative buffy coat techniques against microscopy?
2. How was the evaluation of performance characteristics of rapid diagnostic technique using CareStart™ malaria cassette and quantitative buffy coat against microscopic technique in malaria parasite screening?
3. What was the level of agreement between rapid diagnostic and quantitative buffy coat techniques against microscopy technique in malaria parasite screening?

1.5 Study Objectives

The study was guided by the following objectives.

1.6 General Objective

The main objective of this study was to evaluate the prevalence of transfusion transmitted malaria among blood donors and evaluate Carestart™ and quantitative buffy coat methodologies at Kenyatta National Hospital.

1.7 Specific Objectives

The specific objectives of this study were:

1. To determine the prevalence of malaria parasites among blood donors using rapid diagnostic and quantitative buffy coat techniques and rapid diagnostic against microscopy.
2. To evaluate the performance characteristics of rapid diagnostic technique using CareStart™ malaria cassette and quantitative buffy coat against microscopic technique in malaria parasite screening.
3. To determine the level of agreement between rapid diagnostic and quantitative buffy coat techniques against microscopy technique in malaria parasite screening.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Transfusion Transmitted Malaria.

Transfusion

transmitted malaria is emerging as a major public health issue, particularly in the developing countries, those falling in the zone of endemicity. Transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion transmitted infection (Kitchen & Chiodini, 2006). Since then, a number of different infections have been reported to be transmitted through transfusion; however, still malaria remains as one of the most common TTIs on the global scale. In malaria endemic countries, the issue is far greater as the majority of donors may be potentially infected (Kakkilaya, 2003; Kitchen & Chiodini, 2006). In addition, it is important to indicate that malarial parasites are stable in plasma and whole blood for at least 18 days when stored at 4°C and for extended periods in frozen state (Garfield, Ershler, & Maki, 1978; WHO, 2010).

Morbidity and mortality associated with blood donations contaminated with malaria parasites have been well established in the emerging economies (Borkow & Doucoure, 2008). Since malaria is a principal cause of illness and death in Africa (Karunamoorthi, 2012; Karunamoorthi *et al.*, 2013). It represents a major blood borne infectious disease. High prevalence of TTI infections have been observed by means of blood donation (Tayou, Mbanya, Garraud, & Lefrère, 2007). The transfusion transmitted malaria parasite is a life threatening danger to patients already suffering to survive because of illness and disorders and may develop severe complications and fatality due to deficiency of immunity (Lacerda *et al.*, 2014).

The recently obtained data show that the incidence of TTM varies from under 0.2 in non-endemic countries to 50 or more cases per million in endemic countries.

The whole blood and red blood cell (RBC) units are the primary carriers of TTM though platelets and leukocytes may contain variable numbers of RBCs and have seldom transmitted malaria. Malaria parasites can survive in donated blood between 2 and 6°C for 3 weeks (Chattopadhyay, Majum, & Kumar, 2011; Grand, Seed, Kitchen, & Davis, 2005). The health problems posed by TTM are immense, especially when the blood donation is obtained without proper blood management strategies.

2.2 Prevalence of Transfusion Transmitted Malaria

A recent study demonstrated a relatively high likelihood of TTM via transfusion in sub-Saharan African countries, illustrated by a median prevalence of malaria and determined by the evaluation of thick smears of 10.2 percent (range 0.7 percent in Kenya to 55 percent in Nigeria) in donor blood samples (Owusu-Ofori *et al.*, 2013). Guiguemide (Guiguemide *et al.*, 1992) reported that, in Burkina Faso about 14 percent of donors were infected with *P. falciparum* and higher densities and infectivity of malaria parasites among the silent carriers under 55 years old. A study carried out by Ali, Gader, Kadaru, & Mustafa (2004) in Sudan established that the prevalence of infected donors was 6.5%, the majority of them were between 20 and 40 years old. In endemic countries, distinguishing cases of TTM from natural infections is quite a challenging task, as malaria, occurring post transfusion, can be either a result of a natural infection or transfusion. Thus, the incidence of TTM in malaria endemic countries is indisputably underreported (Brouwer *et al.*, 2013).

Transfusion of blood products in the vulnerable neonates need to be strictly regulated in order to circumvent the intrinsic risks of transmission of avoidable infections particularly malaria (C. K. Murray, Gasser, Magill, & Miller, 2018; N. A. Murray & Roberts, 2004). Notably, the incidence of TTM is very low in non-endemic countries as a result of strict implementation of donor selection as well as screening practices (Brouwer *et al.*, 2013).

2.3 Implications of Transfusion Transmitted Malaria infection

TTM can be associated to serious implications in terms of laboratory medicine and healthcare management. These includes: first, a febrile transfusion reaction can evasively simulate a hemolytic transfusion reaction, second, it could trigger emergence and spread of drug resistant malarial parasites, third, it affects all age groups from newborns to the geriatric group, and fourth, the immunocompromised (cancer and leukemia therapy) patients are at a high risk when the units are not effectively screened for malarial parasite (Bahadur, Pujani, & Manjula, 2010).

The administration of anti-malarial to transfusion recipients may help to avert transmissions. Nevertheless, no matter what strategies are adopted, TTM may still occur. Therefore, a patient with a febrile illness of post-transfusion must always be suspected as malaria in malaria prone settings (Kitchen & Chiodini, 2006). However, if it occurs in non-endemic countries, both the patient as well as donors' case history must be thoroughly investigated and treated accordingly. Therefore, it is recommended to adopt both rapid detection devices with peripheral smears screening of positive cases and with reasonably most reliable methods to prevent TTM (Bahadur *et al.*, 2010).

2.4 Screening for Transfusion Transmitted Malaria

Malaria is one of the preventable and curable illnesses. A key to effective malaria case management often heavily relies on prompt diagnosis and treatment (Karunamoorthi, 2012; Karunamoorthi & Bekele, 2009; Karunamoorthi *et al.*, 2013). The ability to screen blood donations, as well as donors, can significantly decrease risk of TTM.

Laboratory screening for malaria parasite remains the possible option for reducing transfusion of malaria. There are four specific targets for donation screening: intracellular parasites, plasmodial antibodies, plasmodial antigen, and plasmodial DNA. Precise diagnosis precludes unnecessary usage of antimalarial, particularly emergence of antimalarial-resistant strains, cost of treating malaria as well as considerable morbidity and mortality (N. A. Murray & Roberts, 2004).

2.5 Qualities of a good Transfusion Transmitted Malaria screening tool

Major characteristics of a useful malaria diagnostic tool include first the ability to definitively establish presence or absence of infection, second to determine which malaria species is present, third quantifying parasitemia (parasites per microliter of blood or percent red blood cells infected), fourth detecting low level parasitemia, and fifth allow the monitoring of response to antimalarial therapy (including detection of recrudescence or relapse) (WHO, 2010, 2011). Test characteristics that are important for diagnosis vary depending on the epidemiology of infection and goals for control in the region where the test is used (Bell & Peeling, 2006).

2.5.1 Performance characteristics of a malaria parasite screening tool.

The basic performance characteristic of a test designed to distinguish infected from uninfected individuals are sensitivity, that is the probability that a truly infected individual will test positive and specificity, that is the probability that a truly uninfected individual will test negative. These measures are usually expressed as a percentage. Two other important measures of tests performance are positive predictive value (PPV), the probability that those testing positive by the test are truly infected, and negative predictive value (NPV), the probability that those testing negative are truly uninfected. Both of these measures are often expressed as percentages. PPV and NPV depend not only on the sensitivity and specificity of the test, but also on the prevalence of infection in the population studied (Banoo *et al.*, 2006; WHO, 2015; WHO & TDR, 2010).

2.5.2 Operational Characteristics.

Operational characteristics include the time taken to perform the test, its technical simplicity or ease of use, user acceptability and the stability of the test under user conditions. The ease of use will depend on the ease of acquiring and maintaining the equipment required to perform the test, how difficult it is to train staff to use the test and interpret the results of the tests correctly and stability of the test under the expected conditions of use (Banoo *et al.*, 2006; Seed *et al.*, 2005; WHO, 2015).

2.6 Types of diagnostic assays

2.6.1 Syndromic diagnosis

One widely used method to diagnose malaria is syndromic diagnosis, in which the diagnosis is made on the basis of clinical history, signs and symptoms. In many endemic areas without

adequate diagnostic capacity, patients with a febrile illness are likely to receive the diagnosis of malaria. There are a number of pitfalls associated with this approach. First, there is significant clinical overlap among febrile illnesses; fever alone is too nonspecific to make any particular diagnosis. Second, co-infections can and do occur, and treatment for one obviously does not treat the other. Third, malaria parasitemia can occur that is not the cause of the febrile illness. Last, relying on clinical diagnosis alone results in treatment of patients with anti-malarial drugs for illnesses other than malaria. The WHO recommends against this practice when and where malaria diagnostic tests are available (Okocha, Ibehb, Elec, & Ibeha, 2005; WHO, 2009a).

2.6.2 Routine microscopy

Laboratory diagnosis of malaria is usually done through microscopic examination of thick and thin smears (Shah *et al.*, 2004). Thick smears are highly sensitive in detecting malaria parasites as the greater volume of blood is to be examined, whereas thin smears aid in species identification (CDC, 2004). Microscopy permits not only the determination of the species but also the circulating parasite stage too. Though microscopy remains to be a gold standard technique for decades, it demands for adequate technical skill and manpower. It spurred the development of several non-microscopic diagnostic tools and techniques like malaria rapid detection tests (MRDTs).

In most endemic areas, microscopic slide examination of peripheral blood remains the most widely used test as well as the gold standard for detecting malaria parasitemia (Liu *et al.*, 2016). Up to now, there is no evidence on best guidance to indicate which malaria screening methods are effective by transfusion services. WHO (2012) indicates that estimates of diagnostic sensitivity of microscopic slide evaluation vary according to the type of infecting species,

geographical area, and other factors, but in general diagnostic sensitivity is thought to be no higher than 75%. This figure is based on the rate of detection of parasitemia in patients with clinical malaria. For patients with non-falciparum malaria, low level parasitemia, or partial immunity, or those who have been partially treated for malaria, the diagnostic sensitivity is likely to be even lower than 75%. Even so, microscopy offers significant advantages over other methods, and, where it can be done correctly and with good quality assurance, it remains the gold standard against which other methods are compared. Microscopy is based on examination of both thick and thin films made from the same sample of peripheral blood. The diagnostic advantages of routine microscopy are that first it permits definitive identification of infecting species as well as mixed infections; secondly it can be used to determine the magnitude of parasitemia; thirdly it can be used for serial examinations to monitor the efficacy of therapy; fourthly it requires little laboratory infrastructure; fifth it is comparatively inexpensive. Finally, it can also be performed to diagnose various other infectious diseases like tuberculosis and sexually transmitted diseases. Besides, smears provide a table record for further quality assessment, if needed (Jayagowri, Shubhangi, & Vimal, 2014). Microscopic slide examination does have diagnostic disadvantages, including that first and foremost it does not detect very low parasitemias; second, errors in interpretation are most common with either very low or very high parasitemias; third, mixed infections are often missed. Lastly, it is not as useful in areas without endemic malaria because of the inability of persons reading smears to remain sufficiently competent to make accurate and reproducible diagnoses. Though microscopy remains to be a gold standard technique for decades, it spurred the development of several non-microscopic diagnostic tools and techniques like malaria rapid detection tests (MRDTs).

2.6.3 Malaria Rapid Diagnostic Tests (RDTs)

Malaria Rapid Diagnostic Tests (RDTs) are used increasingly for diagnosis of malaria, particularly in remote tropical areas where good microscopy based diagnosis is impractical. RDTs must therefore be robust, simple and safe to use, and reliably demonstrate when malaria parasitemia is and is not present (WHO, 2012). MRDTs are primarily based on the detection of specific malarial antigen in the whole blood (Bahadur et al., 2010) and are also known as Dip sticks or Malaria Rapid Diagnostic Devices. The test involves capture of monoclonal antibody applied to a strip of nitrocellulose which acts as the immobile phase.

The migration of the antigen antibody complex in the mobile phase along the strip enables the labeled antigen to be captured by the monoclonal antibody of the immobile phase, thus producing a visible colored line. Migration depends on several physical characteristics of the component reagents, primarily the porosity of the membrane controlling the flow rate and the components of the buffer solution used to transport the labeled antigen antibody complex in the lysed blood sample. RDTs are an alternative to microscopic diagnosis, particularly wherever good quality microscopy services are not readily available (WHO, 2015). Some MRDTs can detect only one species *Plasmodium falciparum*; however, a few of them can detect three other human species. This includes: *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Though the effectiveness of RDTs under field conditions heavily relies on the quality of test, it is one of the affordable idyllic techniques to improve the blood safety chain.

It is extremely helpful wherever conventional diagnostic tools and techniques are impossible and impracticable (Pruett *et al.*, 2015). The following major drawbacks have been observed with the MRDTs: first result is qualitative and second it is unable to detect the infection when the

malaria parasites are few in number. Consequently, all the negative MRDTs results must be confirmed with microscopy (CDC, 2004; Liu *et al.*, 2016). Besides, microscopy is extremely imperative to quantify the percent of infected RBC, which is a key prognostic indicator.

2.6.4 Quantitative Buffy Coat Method (QBC)

The Quantitative Buffy Coat Method (QBC) Test, developed by Becton and Dickenson in 1983, using a capillary tube pre-coated with acridine orange for the rapid quantification of leucocytes in peripheral blood by staining all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope (Spielman *et al.*, 1988). This technique was then successfully applied for the diagnosis of malaria as the acridine orange staining permits differential coloration of green (nucleic) and red (cytoplasm) in stained parasites. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under UV light source (Spielman *et al.*, 1988).

The parasite nuclei fluoresce bright green, and the cytoplasm appears yellow-orange. The key feature of the method is centrifugation and thereby concentration of the red blood cells in a predictable area of the QBC tube, making detection easy and fast. Red cells containing Plasmodia are less dense than normal ones and concentrate just below the leukocytes, at the top of the erythrocyte column (Mungai *et al.*, 2001). The float forces all the surrounding red cells into the 40-micron space between its outside circumference and the inside of the tube. Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright specks of light among the non-fluorescing red cells. Virtually all of the parasites found in the 60 microliter of blood can be visualized by rotating the tube under the microscope. QBC technique has the advantage of being fast, easy and sensitive. The technique has the

disadvantages of being costly, requiring technical skills, identification of the species and quantification of parasitemia are difficult (Kakkilaya, 2003).

2.6.5 Recommendations for screening of TTIs by WHO

WHO recommends that, first each country should have a national policy on blood screening that defines national requirements for the screening of all whole blood and apheresis donations for transfusion-transmissible infections. Second, that there should be a national programme for blood screening which sets out the strategy facility. Third that, all whole blood and apheresis donations should be screened for evidence of infection prior to the release of blood and blood components for clinical or manufacturing use (Kakkilaya, 2003; Talib & Khuana, 1996). Fourth that, screening of all blood donations should be mandatory for the following infections and using the markers for one, HIV-1 and HIV-2: screening for either a combination of HIV antigen-antibody or HIV antibodies, two Hepatitis B: screening for hepatitis B surface antigen (HBsAg) three, Hepatitis C: screening for either a combination of HCV antigen antibody or HCV antibodies and four Syphilis (*Treponema pallidum*): screening for specific treponemal antibodies (WHO, 2009b, 2010).

Further WHO emphasizes that screening of donations for other infections, such as those causing malaria and *chagas* disease should be based on local epidemiological evidence. Where feasible, blood screening should be consolidated in strategically located facilities at national and/or regional levels to achieve uniformity of standards, increased safety and economies of scale. Adequate resources should be made available for the consistent and reliable screening of blood donations for transfusion transmissible infections. A sufficient number of qualified and trained staff should be available for the blood screening programme. There should be a national system

for the evaluation, selection and validation of all assays used for blood screening (Adewuyi, 2001; Tayou *et al.*, 2007).

The minimum evaluated sensitivity and specificity levels of all assays used for blood screening should be as high as possible and preferably not less than 99.5%. Quality assured screening of all donations using serology should be in place before screening strategies utilizing nucleic acid testing are considered. There should be a national procurement policy and supply system to ensure the quality and continuity of test kits, reagents and other consumables required for the screening of all donated blood. Quality systems should be in place for all elements of the blood screening programme, including standards, training, documentation and assessment (Banoo *et al.*, 2006.)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Kenyatta National Hospital, Blood Transfusion Unit. The Kenyatta National Hospital is the largest referral hospital providing specialized healthcare to over 30 million of the country's population in addition to other referral clients from East and Central African. In order to meet the constant blood demand at the hospital, the Blood Transfusion Unit is tasked with managing the hospital's supply of safe blood and its components. The Blood Transfusion Unit was created to meet the needs of the hospital and address blood donation gaps. If the hospital was to solely rely on Kenyatta National Blood Transfusion Unit to provide blood and its products, the supply at the hospital would further decrease.

3.2 Study Design

This was a descriptive cross sectional study. This was because it described or found out the percentage prevalence of malaria parasite among blood donors. This type of study involved collection and gathering of quantitative data about blood donors who suffered or did not suffer from malaria infection. It used visual aids such as graphs, and tables. This descriptive study was dependent on instruments for measurement and observation. These instruments for measurement and observation included microscopes, and centrifuge so that the resulting measurement were able be accurate, reliable and generalizable. This kind of descriptive study produced statistical information about aspects of implementing screening malaria parasite and provide valuable insights to practitioners, researchers and policy makers. This study described,

explained and validated findings. It was also a cross sectional study because it observed a defined population at a single point in time.

3.3 Study Population

The population of the present study was composed of men and women of 17 years and above who voluntarily visited Kenyatta National Hospital, Blood Transfusion Unit for the purpose of donating blood.

3.4 Study Equipment and Other Consumables

The equipment and consumables that were used in this study included: ordinary microscope which was used for microscopic examination of malaria parasite, florescent microscope for examining florescing of the parasite, haematocrit centrifuge which was used for spinning blood contained in capillary tubes, refrigerator set between 2- 8⁰C for storage of blood samples.

Other consumable materials were vacutainer tubes for aliquoting blood samples for analysis, microscope slides for holding blood samples, staining jars for putting Giemsa stain and the fixative, absolute methanol for fixing thick blood smears, drying racks for drying thick blood smears, latex gloves for hand protection , cotton wool, gauze for wiping the back of microscope slides, waste disposal containers for disposing off laboratory waste which will be generated during the study, normal saline for rinsing Pasteur pipettes, microscope tissue cleaning lenses for cleaning microscope lenses, laboratory coat for protecting the investigator, quantitative buffy coat capillary tubes for holding blood samples, malaria strips for detection of malaria parasite, buffer as a diluent for stain, Pasteur pipettes which were used for picking blood samples, methylated spirit which was used as a disinfectant during the study, oil immersion for

improving refractive index during microscopic examination and Giemsa stain for staining purposes.

3.5 The Inclusion Criteria

The inclusion criteria involved selection of blood donors labelled using specific laboratory numbers non haemolyzed and whole blood samples.

3.6 The Exclusion Criteria

The exclusion criteria involved blood donors without specific labels using laboratory numbers, clotted blood samples, separated donors blood and haemolysed donors blood.

3.7 Sample Size Determination

The sample size was calculated using the Fischer et al.,(1998) formulacited in Waithaka et al., (2010).

$$n = \frac{Z^2 \times p \times (1 - p)}{e^2}$$

Where

n was the sample size required

z was the value of standard variate at a given confidence level (z = 1.96)

p was the probability of prevalence of malaria parasitaemia among African donors is 10.2 %(Garba, Jatau, Inabo, Aminu, & Mamman, 2009; Ikeh & Okeke, 2006).

1-p was the probability of non-prevalence of malaria parasitaemia amongst African donors

e was the precision level at 95 percent confidence interval (e = 5 %)

$$n = \frac{1.96^2 \times 0.102 \times (1 - 0.102)}{0.05^2}$$

$$n = 140.75$$

$$n \approx 141$$

The sample size was increased by a factor of 10 percent in order to cover up for any discrepancies and attrition of the study participants. So n =155

$$n_t = n \times 110 \% ; n_t = \frac{141 \times 110}{100} ; n_t = 155$$

3.8 Sampling Method

The sample was obtained on convenience basis and the blood donors available at the time of data collection selected for the day.

3.8.1 Laboratory Procedures

3.8.2 Sample Identification

Donor's blood sample was identified from each donor using different laboratory numbers that were given to each sample. These samples were not screened for transfusion transmitted infections.

3.8.3 Sample Collection and Handling

Anticoagulated lavender tubes were labeled using various laboratory log numbers. Then 2mls of blood donations from each donor main bag which contains approximately 450mls of blood was aliquoted. These samples were then transferred in to test tube rack. They were stored at 2°C. Processing of each donor blood sample followed within the same day or at most the following day(M. Cheesbrough, 1998; Monica Cheesbrough, 2008).

3.8.4 Preparation of Thick Blood Film and Staining.

A thick film was made by placing 6µl of donor's blood at the Centre of grease-free microscopic slide. Without delay, the blood was spread with a glass spreader held at a steep angle to achieve a thick smear covering an area of 15 x 15 mm. This was allowed to air dry. Then 10% Giemsa stain was applied on the thick film and allowed for 20 minutes. After this time, the stain was washed off using distilled water and again allowed to air-dry. The dried stained thick smear was viewed under a light microscope using 100x oil immersion objective lens. Results were observed and recorded.

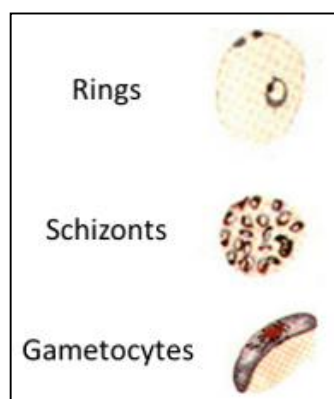


Figure 3. 0: Three stages of malaria parasites:rings,schizonts and gametocytes.

3.8.5 Rapid Diagnostic Test Using Carestart cassette.

Two milliliters of donor's blood were collected in a lavender tube containing EDTA anticoagulant. The pipette was gently squeezed. The blood was touched with the open end of the pipette. The pipette was gently released to aspirate blood up to the black mark. 5µl of blood was added on the sample well labeled S. The tab was twisted and pulled to open the assay buffer. 2 drops of buffer were added on the buffer well labeled A. Results were taken after 20 minutes.

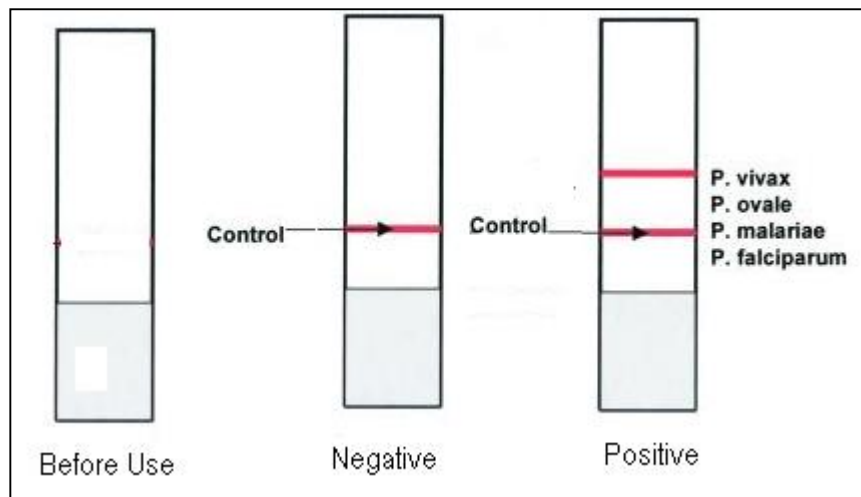


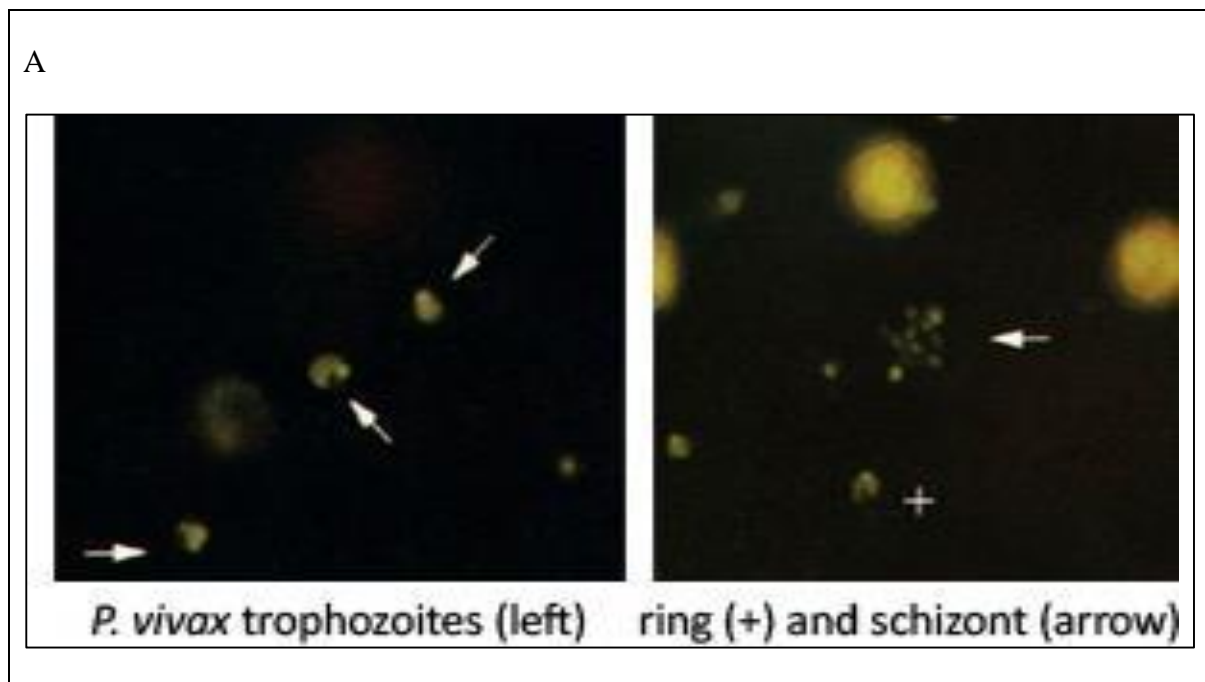
Figure3.1: Expected results for Rapid Diagnostic Test Using Strip Method

3.8.6 Quantitative Buffy Coat

The Quantitative Buffy Coat (QBC) tube is a high-precision glass hematocrit tube, pre-coated internally with acridine orange stain and potassium oxalate. Acridine orange (AO) binds deoxyribonucleic acids and ribonucleic acids. The malaria parasite binds acridine orange in the nucleus and the cytoplasm and emits green and red fluorescence when excited at 480 nm allowing the detection and examination of parasite morphology by fluorescent microscopy.

The following procedure was used in the QBC technique. The tube was filled with 60 microliters of blood. A clear plastic closure was then attached. A precisely made cylindrical float, designed to be suspended in the packed red blood cells, was inserted. The tube was centrifuged at 12,000 rpm for 5 minutes. The components of the buffy coat were separated according to their densities, forming discrete bands.

The QBC tube was placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, using X60 microscope objective. Fluorescing parasites were observed at the red blood cell or white blood cell interface (Buffy Coat). A negative test was reported within one minute and positive result within minutes.



B



P. falciparum double rings (left) and single ring

C



P. vivax gametocyte (left) and *P. falciparum* gametocytes

Figure3.2: Quantitative Buffy Coat Images of *Plasmodium* parasites. *P. vivax* gametocyte (Panel A) and *P. falciparum* gametocytes

3.8.7 Quality control samples, identification and handling

Test control samples were run with every new batch of test samples. Approximately 2mls of blood sample positive for malaria infection was out sourced within clinics in Nairobi and collected using anticoagulated vacutainer tube contained in a rack. The sample was transported using a cool box up to its destination for processing. Negative blood sample was obtained from a patient who were known to have not suffered from malaria infection. Approximately 2mls of blood sample identified negative for malaria infection was out sourced and collected using anticoagulated vacutainer tube contained in a rack. This sample was transported using a cool box up to its destination for processing. Results were observed and interpreted.

3.8.8 Determination of prevalence of transfusion transmitted malaria parasite

The prevalence transfusion transmitted malaria among donors was determined using the following formula: -

$$P = \frac{BTP}{PBD} \times 100(\text{CDC, 2004})$$

Where: -

P – Prevalence

BTP - Blood donors tested positive for malaria parasite

PBD - Population of blood donors

3.9 Evaluating the Performance Characteristics of RDT and QBC Techniques against Microscopy Technique in Malaria Parasite Screening.

3.9.1 Sensitivity, Specificity and Predictive Values

Sensitivity is defined as the probability that the sample will be positive when the infection is present while specificity is the probability that the sample will be negative when the infection is absent. The (PPV) is the probability that those testing positive by the test are truly infected, and negative predictive value (NPV), the probability that those testing negative are truly uninfected. Both of these measures are often expressed as percentages.

Data was entered in 2×2 contingency tables and analyzed for performance characteristics using the formulas (Petra *et al*, 2010).

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100 \%$$

$$PPV = \frac{TP}{TP + FP}$$

$$NPV = \frac{TN}{FN + TN}$$

Where,

PPV - Positive predictive value

NPV - Negative predictive value

TN - true negative,

TP - true positive,

FN - false negative and

FP - false positive.

3.9.2 Determining the Level of Agreement Between RDT and QBC Techniques against Microscopy Technique in Malaria Parasite Screening.

The level of agreement among the malaria screening techniques was determined using the Kappa statistics. Reliability was established using kappa statistic to determine the extent to which the two screening methods agree with the gold standard beyond what was expected to be observed based on chance alone. A kappa of 1 indicated perfect agreement, whereas a kappa of 0 indicated agreement equivalent to chance. The following kappa formula was used:

$$K = \frac{P_o - P_e}{1 - P_e}$$

Where

K – Kappa

P_o – Observed agreement

P_e –Expected agreement is given by; $pe = \left(\frac{n_1}{n} \times \frac{m_1}{n}\right) + \left(\frac{n_0}{n} \times \frac{m_0}{n}\right)$

The observed agreement (P_0) was be calculated as follows:

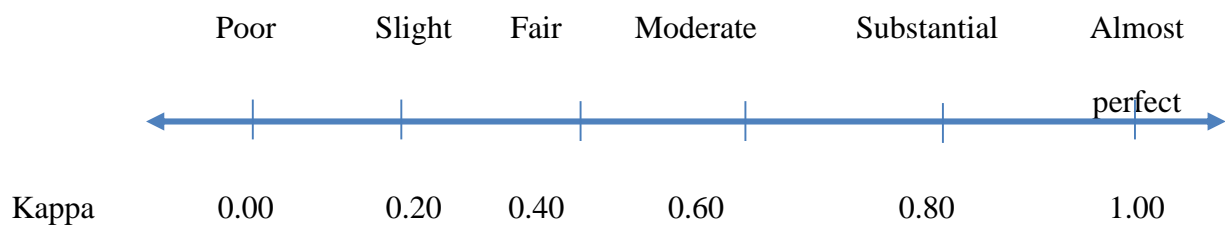
$$P_0 = \frac{a + d}{N}$$

Table 3.1: Agreement scoring table

		Screening technique I		
		Positive	Negative	Total
Screening technique II	Positive	a	B	m ₁
	Negative	c	D	m ₀
Total		n ₁	n ₀	N

Where (a) and (d) represent the number of times the two screening methods agree while (b) and (c) represent the number of times two techniques disagree. If no agreement (b) and (c) would be zero and observed agreement (p_0) is 1 or 100%. If there are no agreements, (a) and (d) would be zero, and the observed agreement (p_0) is 0.

Table 3.2: Interpretation of Kappa



Legend

Kappa	Agreement	Kappa	Agreement
<0	Less than chance agreement	0.41-0.60	Moderate agreement
0.01-0.20	Slight agreement	0.61-0.80	Substantial agreement
0.21-0.40	Fair agreement	0.81-0.99	Almost perfect agreement

3.10 Limitations of the Study

The research process required considerable amounts of finances which included hire of laboratory equipment, materials and sundries. The study was conducted at a time when the country was facing a series of national health workers' industrial actions including go-slows, strikes and peaceful demonstrations which turned out to be a challenge and prolonged the study period.

3.11 Data Analysis and Presentation

Data captured, analysed and presented in tables. 2x2 contingency tables were used for kappa analysis. The kappa test was used to determine the level of agreement of rapid diagnostic and quantitative buffy coat techniques. Microscopy was used as the gold standard.

3.12 Ethical considerations

High value of knowledge was gained through this research. The researcher recognized that obtaining a valid sample from voluntary blood donors would entail obtaining informed consent from each donor. In order to conduct the study, the researcher submitted a written request to

the Director, Laboratory Medicine at Kenyatta National Hospital seeking permission to conduct the study with the blood donations at the Blood Transfusion Units. All sample donations found to be positive for malaria parasite were reported to the laboratory in-charge, Blood Transfusion Unit in order to give advice on them. Ethical approval was sought and granted by Kenyatta National Hospital - University of Nairobi Ethics and Research Committee under reference number **KNH-ERC/Mod& SAE/232**.

CHAPTER FOUR

RESULTS

4.1 Demographic Data Analysis

This cross sectional study was conducted in Kenyatta National Hospital Kenya on 155 voluntary replacement blood donors who presented them in the study without any sign of illness between March to May 2017.

Table 4.0: Donors screened for malaria parasites with respect to age and sex

Age	Males Screened %	Females screened %
10-19	4.7(5)	6.3(3)
20-29	62.6(67)	52.1(25)
30-39	26.2(28)	35.4(17)
40-49	6.5(7)	4.2(2)
50-59	0.0 (0)	1(1)
Total	100	100

Table 1.1: Donors tested positive for Malaria Parasites using microscopy with respect to age and sex.

Age	Males Positive %	Females Positive %
10-19	0.0	0.0
20-29	0.0	0.0
30-39	4.7(5)	0.0
40-49	0.0	0.0

50-59	0.0	0.0
Total	4.7	0.0

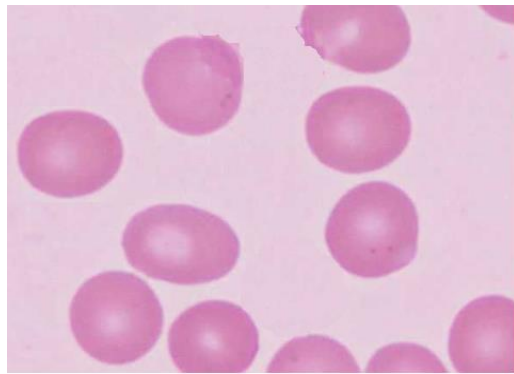


Figure 4.0: Panel A shows Malaria parasites Negative using microscopy

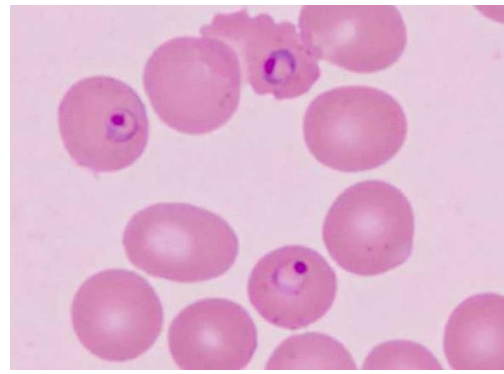


Figure 4.1: Panel B shows Malaria Parasites Positive using microscopy



Figure 4.2: Panel A shows Malaria Parasites Negative using RDT



Figure 4.3: Panel B shows Malaria Parasites Positive using RDT

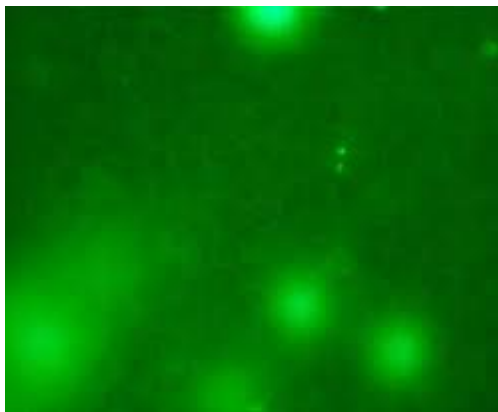


Figure 4.4: Panel A Malaria Parasites Negative using QBC

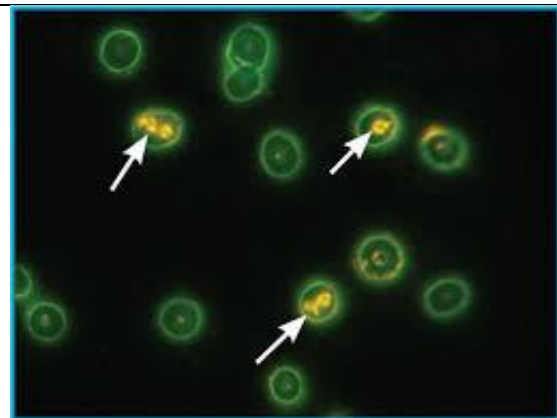


Figure 4.5: Panel B shows Malaria Parasites Positive using QBC

4.2 Prevalence of malaria parasite among blood donors.

Table 4.2: Prevalence of malaria parasites among blood donors.

Technique	Samples Tested Positive for malaria parasites	Prevalence (%)
Microscopy	5	3.2
Rapid Diagnostic Test	8	5.2
Quantitative Buffy Coat Technique	6	3.9

The point prevalence of malaria parasites among blood donors was determined using rapid diagnostic technique and quantitative buffy coat and they were compared against microscopy. Microscopy technique reported a prevalence of 3.2%, Rapid diagnostic technique 5.2% and quantitative buffy coat reported prevalence of 3.9 % as shown on the table 5above. The prevalence of blood donors who were infected by malaria parasites using the three techniques was arrived as show below. Microscopy-blood donors truly affected by malaria parasites were 5, population of blood donors were 155 then multiplied by 100.Rapid diagnostic technique-blood donors truly affected by malaria parasites were 8, population of blood donors were 155 then multiplied by 100.Quantitative Buffy Coat technique-blood donors truly affected by malaria parasites were 8, population of blood donors was 155 then multiplied by 100.Performance characteristics of rapid diagnostic and quantitative buffy coat techniques against microscopy technique.

Table 4.3: Evaluating the performance characteristics of two malaria screening techniques. Microscopic technique was used as the gold standard.

Malaria Screening Technique	Performance characteristics			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Microscopy	100	100	100	100
QBC	100	99	83	100
RDT	80	97	50	99

The sensitivity of rapid diagnostic test and quantitative buffy coat was, 80% and 100% respectively. The specificity of these malaria infection screening tools was reported to be 99% and 97% respectively. The positive predictive values for rapid diagnostic test and quantitative buffy coat was reported to be 50 % and 83% respectively. The negative predictive values reported for the screening techniques was 99% and 100 % respectively. Sensitivity, specificity, positive predictive and negative predictive values were arrived as shown (Table 4.3).

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Sensitivity}_{RDT} = \frac{4}{4+1} \times 100 = 80\% ; \text{Sensitivity}_{QBC} = \frac{5}{5+0} \times 100 = 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100 \%$$

$$\text{Specificity}_{RDT} = \frac{146}{146+4} \times 100 \% = 97\% ; \text{Specificity}_{QBC} = \frac{149}{149+1} \times 100 \% = 99\%$$

$$PPV = \frac{TP}{TP + FP} \times 100$$

$$PPV_{RDT} = \frac{4}{4+4} \times 100 = 50\% ; PPV_{QBC} = \frac{5}{5+1} \times 100 = 83\%$$

$$NPV = \frac{TN}{FN + TN} \times 100$$

$$NPV_{RDT} = \frac{146}{1+146} \times 100 = 99\% ; NPV_{QBC} = \frac{149}{0+149} \times 100 = 100\%$$

Table 4.6: Summarized Computed Characteristics of Screening Techniques

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RDT	80	97	50	99
QBC	100	99	83	100

4.3 Determining Level of Agreement between Rapid Diagnostic Test and Quantitative Buffy Coat against the Microscopy Technique.

Table 4.7: Determining the Level of Agreement between Rapid Diagnostic Technique and Microscopy Technique.

Tests		Microscopy Technique- Gold Standard		
		Positive	Negative	Total
RDT Technique	Positive	4	4	8
	Negative	1	146	147
Total		5	150	155

$$\kappa = 0.63$$

The level of agreement between rapid diagnostic technique and quantitative buffy coat against microscopy technique was determined using Kappa statistic. A 2×2 contingency table was used to tabulate the results. The kappa value resulted to 0.63.

Table 4.8: Determining the level of agreement between Quantitative buffy coat and microscopy technique.

Tests		Microscopy Technique - Gold Standard		
		Positive	Negative	Total
QBC	Positive	5	1	6
Technique	Negative	0	149	149
Total		5	150	155

$$K = \frac{P_0 - P_e}{1 - P_e} \quad P_0 = \frac{a+d}{N} = \frac{5+149}{155} = 0.9935 \quad p_e = \frac{5 \times 6}{155 \times 155} + \frac{150 \times 149}{155 \times 155} = 0.932$$

$$K = \frac{0.9935 - 0.932}{1 - 0.932}$$

$$\kappa = 0.90$$

The level of agreement between quantitative buffy coat and microscopy was determined using Kappa statistic. A 2x2 contingency table was used to tabulate the results as shown in the table above 8. Microscopic technique was used as the gold standard. The kappa value resulted to 0.90.

Table 4.9: True positive, true negative, false positive and false negative values of blood donor samples analysed using the two techniques against microscopy technique.

Technique	TEST RESULTS			
	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)
Microscopy	5	150	0	0
Rapid Diagnostic Test	4	146	4	1
Quantitative Buffy Coat	5	149	1	0

Microscopy which was used as the gold standard in malaria infection screening reported 5 true positives (TP), 150 true negatives (TN), 0 false positives (FP) and 0 false negative (FN) cases. Rapid diagnostic test reported 4 true positives (TP), 146 true negatives (TN), 4 false positives (FP) and 1 false negative (FN) cases. Quantitative buffy coat technique reported 5 true positives (TP), 149 true negatives (TN), 1 false positive (FP) and 0 false negative (FN) cases as tabulated in table 4.9 above.

CHAPTER FIVE

DISCUSSION

This present study aimed at determining the prevalence of malaria parasites among blood donors using rapid diagnostic and quantitative buffy coat techniques against microscopy, evaluation of the performance characteristics of rapid diagnostic technique using CareStart™ malaria cassette and quantitative buffy coat against microscopic technique in malaria parasite screening and determining the level of agreement between rapid diagnostic and quantitative buffy coat techniques against microscopy technique in malaria parasite screening.

The transmission of malaria infection through blood transfusion is one of the oldest recorded incidents of transfusion transmitted infections in transfusion facilities due to lack of screening. Malaria transmissions associated with blood transfusion in malaria prone zones has been on the rise. This study has confirmed that blood donors that are seen at Kenyatta National Hospital have a prevalence of 3.2%. Based on observations from malaria screening questionnaire directed to asymptotology of blood donors; this study has showed that voluntary blood donors contribute to the risk of transfusion transmitted malaria in low and high prevalence zones. While Nairobi is classified as low malaria endemic zone transmission of malaria through blood transfusion is still present. A study by Kitchen et al., (Kitchen et al., 2005) which showed that transmission of malaria infection by blood transfusion is a possible cause of malaria infections among blood donors and it agrees with this study. Other similar studies carried out including a study carried out in Abakaliki Metropolis obtained a prevalence of 40.9% among blood donors which was high contrary to this study (Epidi et al., 2008). The difference in those studies and this study could have been caused by differences in sample size used, endemicity, period during which the data was collected and the technique used for diagnosis. While a lower

malaria prevalence of 1% was reported in Ethiopia among blood donors this study was comparable to other studies that were done in Ghana (10%), Sudan (5%) and Yaoundé (6.5%) (M. S. M. Ali et al., 2004; Noubouossie et al., 2012; Owusu-Ofori et al., 2013).

In this study, the prevalence of malaria infection among blood donors was found to be 3.2% by microscopy, Quantitative Buffy Coat 3.9 % while the rapid diagnostic test gave a prevalence of 5.2 %. Quantitative Buffy coat technique results compared well with microscopy because it picked all positives picked by the gold standard. Rapid Diagnostic Test yielded higher prevalence values above the gold standard because it picked more false positive values than microscopy. RDT was found to detect more false positives than QBC and microscopy. This means that RDT is more prone to false positives than the gold standard. This could have been attributed by confounders such drug resistance, persistence of antigens due to sequestration, incomplete malaria treatment, cross reaction with autoantibodies such as rheumatoid factor and presence of histidine rich protein 2 antigen of plasmodium parasite in the blood of the donor gene deletion or mutation of Histidine Rich Protein-2 (HRP-2) as postulated by Wellems, Walker-Jonah & Panton, (1991) and agreed with a study done by Latha, (2016). The RDT was less accurate in determination of malaria prevalence at KNH compared to the QBC.

The prevalence of malaria parasite infection in blood donor units using QBC and RDT against microscopy obtained in this study was low, although not insignificant. This low prevalence may be accounted for by the study period which was a low and rainy season and there was no much travelling of people from plasmodium endemic region, hence low transmission rates of malaria. The timing of the study from March to May, and the duration of three months, did not cover the peak of long rainfall period of October to December. This timing could have

influenced the prevalence of malaria in the population. Malaria transmission is favoured by the availability of stagnant surface waters that are abundant during the rainy season; these act as breeding sites for the vector mosquitoes of the infection. Another factor influencing the study's low donor prevalence could be the relatively small sample size of 155, although statistically it is adequate to demonstrate the desired parameter.

However, a larger sample size would have increased the power or accuracy of the study, and the obtained prevalence rate might have been higher. This effect of sample size can be seen in the studies from South-Eastern Nigeria that had large sample sizes and concomitant high prevalence of 40.9% and 30.2% respectively (Okocha et al., 2005; Uneke, Ogbu, & Nwojiji, 2006). On the other hand, the studies from Jos (Ikeh & Okeke, 2006) and Zaria (Garba et al., 2009) with small sample sizes had lower prevalence of 11% and 6.8%, respectively. In another similar study which was done by Douglas D *et al.*, (2016) in Nigeria the prevalence of malaria parasites among blood donors was 7.5% which was also relatively low.

Rapid diagnostic test and quantitative buffy coat showed a sensitivity of 80% and 100% respectively. The sensitivity of quantitative buffy coat compared well with the gold standard. This meets the WHO recommended cut off of 95% (WHO, 2013). In this study, the sensitivity of the two malaria screening techniques indicates that quantitative buffy coat technique was highly sensitive in detection of TTM while rapid diagnostic test was less sensitive. A similar study which was conducted in Cameroon by Kwenti, *et al.*, (2017). RDT showed a sensitivity of (88.0%). The rapid diagnostic test produced lower sensitivity values compared to the gold standard and fell below the recommended values of over 95% as it is required by WHO (WHO, 2009b). This study depicted the specificity of rapid diagnostic test and quantitative buffy coat

as 97% and 100% respectively. A similar study done by Bahadur *et al.*, (Bahadur et al., 2010) in India reported rapid diagnostic test and quantitative buffy coat to have specificities of 100% and 100% respectively.

The specificity of QBC which was 100% from that study agreed strongly with the findings from this study on same technique. The specificity of RDT of 97% agreed favourably with the study which was done in India. The specificity of rapid diagnostic test compared favourably with microscopy and the specificity of QBC compared well because it picked all positive values picked by the gold standard. In this study, the positive predictive value for rapid diagnostic test and the quantitative buffy coat was 83% and 50% while the negative predictive values were 100% and 99% respectively. In a similar study that was done by Latha (2016), sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy. This study agreed with the previous study done by Garba et al., (2009) which compared microscopy (gold standard), QBC and antigen detection test and reported the sensitivity and specificity of QBC as 78.94% and 98% respectively while sensitivity and specificity of RDT were 75% and 100% respectively. The sensitivity of QBC and RDT fell below the WHO sensitivity requirement of 99%. This difference could have been attributed by the size of study population, endemicity and period of data collection. Onankpa, Jiya, Achegbulu, & Airede, (2007) in their study reported the sensitivity and specificity of QBC for detection of Plasmodium was 100%. That study also agreed strongly with the present study. The sensitivity and specificity of RDT for detection of Plasmodium was 96% and 100% respectively when compared with microscopy as gold standard. Another study done by Ibhanebhor, Otobo, & Ladipo, (1996) the sensitivity and specificity of the QBC-test was 99.2% and 72% respectively. A study that was done in India using RDT found the sensitivity

and specificity of 93% and 99% respectively (Bahadur et al., 2010; Jayagowri et al., 2014) while a prospective study carried out in Karachi indicated that the test was 97.0% sensitive and 98.3% specific for the diagnosis of Plasmodium species with a PPV and an NPV of 78.0% and 99.8% respectively. M. S. Ali, Gader, Kadaru, & Mustafa, (2005) using ELISA technique found the sensitivity and specificity to be 89.6% and 100% respectively while M. S. M. Ali et al., (M. S. M. Ali et al., 2004) showed the sensitivity and specificity of 100% and 95.4% respectively for detection of Plasmodium species by using nested PCR. In another study, the PCR method was 100% sensitive and 100% specific (Faruk, Ogunrinde, & Mamman, 2017). The findings of sensitivity and specificity from these studies using different techniques did not have significant differences from the present study.

The level of agreement of quantitative buffy coat and rapid diagnostic techniques against microscopy was determined using Kappa statistic. The study found that the Kappa value for quantitative buffy coat compared to microscopic technique was 0.90. This implied that there was almost perfect agreement between QBC and microscopy technique. The study reported a kappa value of 0.63 between the rapid diagnostic technique and the microscopy. This implies that there was substantial agreement between the gold standard and the RDT. The study findings agreed with the conclusion arrived by Jayagowriet *al.*, (2014) that microscopy and quantitative buffy coat techniques are good tools of malaria screening.

In addition to the performance characteristics of a test it was imperatively important to consider the operational characteristics such as cost (Lacerda et al., 2014). A pack of 25 pieces of Carestart™ cassette costed Kshs 1250, while a pack of 100 pieces of QBC cuvettes costed Kshs 6,000 in Nairobi, Kenya in the year 2019. Therefore, a rough estimate for the cost of running

the RDT (Carestart™) and QBC tests would be Ksh 50 and Ksh 60, respectively. The equipment cost for performing one test for malaria infection using microscopic technique is Kshs20. This meant that in terms of cost, screening for malaria infection using microscopic technique was more cheaper compared to QBC and RDT. This study agreed with (Spielman et al., (Spielman et al., 1988) and WHO, (WHO, 2015) that microscopic technique was more laborious, time consuming and required highly skilled technical staff in identification of the plasmodium species. The study affirmed the recommendation by WHO,(2012, 2013, 2015) that QBC technique was less laborious and fast while RDT could easily be used in remote areas since it was portable, fast, did not require highly skilled technical personnel and it did not require electricity.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study confirmed that the prevalence of Malaria parasites among blood donors that are seen at Kenyatta National Hospital was 3.2% by microscopic technique, quantitative buffy coat 3.9% and rapid diagnostic test 5.2%. This implied that blood donations contained some level of malaria parasites which is a healthy risk to patients under transfusion. The risk can be mitigated by use of high precision technologies in blood screening. The study established that the quantitative buffy coat was such a technique which had a high sensitivity of 100% that compared to the gold standard. The Quantitative Buffy Coat technique resulted to a kappa value of 0.90. This established that QBC had an almost perfect agreement with microscopy technique.

6.2 RECOMMENDATIONS

Transfusion transmitted malaria infection is life threatening ailment. The findings from this study wishes to make the following recommendations for consideration that would enhance the efforts of fighting malaria infection.

- i. All blood donations are subjects of infection with malaria and as a result, all blood should be screened for malaria parasites before transfusing the recipients.
- ii. Quantitative buffy coat technique should be used for screening malaria parasite because of its higher sensitivity and specificity compared to rapid diagnostic technique.

- iii. Further studies should be carried out to find out if there is another malaria parasite screening tool which is also sensitive, specific and cheaper.
- iv. In a view of this research finding there is a need to develop comprehensive national policies for safe blood and its products administration to recipients.

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APPENDICES

APPENDIX I: Preparation of Giemsa Stain

Stock Solution:

1. Dissolve 1g of Giemsa (MERCK) powder into 54 ml of glycerine.
2. Heat this solution at $\sim 60^{\circ}\text{C}$ about 1.30h- 2h. Let cool to room temperature.
3. Add 84 ml of methanol.
4. Let the bottle tightly closed and protected from light for one week.
5. Filter the solution and keep protected from light.

Work Solution:

1. Dilute 1 ml of stock solution in 30 ml of phosphate buffer (6.8 pH).

APPENDIX II: Preparation of Buffer pH 6.8

- Phosphate buffer (Sorensen)

Stock A: 0.2M sodium di-hydrogen orthophosphate (mw 156).

To prepare dissolve 3.12g in 100ml distilled water.

- Stock B: 0.2M di-sodium hydrogen orthophosphate (mw 142).

To prepare dissolve 2.83g in 100ml distilled water.

- For pH 6.8 add 25.5ml of A to 24.5ml of B and make up to 100ml with distilled water.

APPENDIX III: Preparation of Buffer pH 7.2

1. Pour 1 L of distilled or de-ionized water into a flask or beaker.
2. Place one buffer tablet into the flask or beaker.
3. Mix by gentle swirling.
4. Label the bottle of buffered water, and document the procedure in the quality control log-book.
5. Use water buffered to pH 7.2 to prepare the Giemsa working solution.
6. Measure 1 L of distilled or de-ionized water into a flask or beaker.
7. Using small tongs or a tweezer, place one phosphate buffer tablet into the flask or beaker. Be careful not to touch the tablet with your hand.
8. Mix by gently swirling the flask or beaker until the tablet is completely dissolved.
9. Label the bottle of buffered water.

APPENDIX IV: Informed Consent to The Blood Donor.

I am a student at Jomo Kenyatta University of Agriculture and Technology undertaking master's degree in medical laboratory science, Haematology and Blood Transfusion option. I am going to carry out a study on determination of risks of transfusion transmitted malaria parasite among blood donors attending Kenyatta National Hospital, blood transfusion unit. This study will be part of my academic requirement and it will not impose any risks to you. The information that I am going to gather from this study will be treated with utmost confidentiality. Kindly, I am going to issue you with a form that you are going to read through and give some information which is required and then sign. Signing for me this form will mean that you have read and understood well the information which is required and you will voluntarily participate in the study. In case of any question you can contact Principal investigator, Theresia Ndunda using mobile number **0735640944**.

APPENDIX V: Donor's Consent Form

The above details about the study and basis of participation have been explained to me and I solely agree to give permission for use of my specimen for the proposed study. I.....do give informed consent to participate in the study fully aware that no risks will be involved. I am also fully aware that the results from this study will be used for academic purpose.

Participant's signature/Thumb print Date.....

Principal investigator's signatureDate.....

APPENDIX VI: Blood Donor Malaria Screening Questionnaire Form

Personal details

Donors full names **Age**.....

Sex..... Male/Female) **Occupation**.....

Marital Status

Single	Yes/No	Married	Yes/No
--------	--------	---------	--------

Cell phone number.....

Demographic data

County..... **Sub-County**.....

HEALTH QUESTIONNAIRE	Tick the appropriate answer	
1. Are you feeling well and in good health?	Yes	No
2. Have you travel from malaria endemic zone for the last three weeks?	Yes	No
3. Are there many mosquitoes where you stay?	Yes	No
4. Do you sleep under mosquito net daily?	Yes	No
5. Have you had malaria infection for the last three months?	Yes	No
6. Have you had malaria infection for the last six months?	Yes	No
7. Did you seek medical attention incase infected by malaria?	Yes	No
8. Did you take antimalarial drugs?	Yes	No

HEALTH QUESTIONNAIRE	Tick the appropriate answer	
9. Did you finish the antimalarial drugs?	Yes	No
10. Were you tested after medication?	Yes	No
11. Can you be screened for malaria infection?	Yes	No

DECLARATION

I declare that the information I have above is correct.

I understand that my blood will be tested for malaria parasite

Consenter's signature.....**Date**.....

APPENDIX VII: Letter of Introduction

Introduction

My name is Theresia Ndunda, a master's student at Jomo Kenyatta University of Agriculture and Technology. I am pursuing Master of Science degree in medical laboratory science with a speciality in Haematology and Blood Transfusion Science.

I would like to introduce to you my research study that I am doing with the aim of giving relevant information and seek consent to use donors blood sample attending Kenyatta National Hospital, Blood Transfusion Unit.

Research Title: Determination of transfusion transmitted malaria parasite among blood donors attending Kenyatta National Hospital, Blood Transfusion Unit.

Confidentiality

Data which will be obtained from the study will be treated with a lot of confidentiality and no names of blood donors will appear in the study. Identity will involve use of donor register numbers.

Contact information

If you have any question regarding the study, please contact the principle researcher, Theresia Ndunda, Jomo Kenyatta University Of Agriculture and Technology, my mobile number **0735640944**, or my supervisors, Dr. Amos Mbugua phone number 0702961963, Dr. Kibet Shikuku phone number 0720789843 and Dr. Naomi Waiganjo phone number 0722392072.

APPENDIX VIII: Permission to use Specimens from the Blood Transfusion Unit at Kenyatta National Hospital

TheresiaNdunda

Technical University of Kenya

P.O Box 52428-00200

Nairobi

25th July 2016

Acting Director

Laboratory Medicine

Kenyatta National Hospital

P.O Box 20723 -00202

Dear Sir,

RE: Permission to use specimens from blood transfusion unit bank at Kenyatta National Hospital.

I am a student at Jomo Kenyatta University of Agriculture and Technology pursuing master's degree in medical laboratory science, Haematology and Blood Transfusion Science option. I am intending to carry out a study on determination of Risks of transfusion transmitted malaria parasite among blood donors attending Blood Transfusion Unit at Kenyatta National Hospital. While carrying out this study, I will be required to screen for malaria parasite using three techniques namely; Thick blood film, Rapid diagnostic technique by use of malaria strip and quantitative buffy coat.

I have shared and discussed my aspects of the study with Aglean Chelimo, in-charge of blood transfusion unit, Kenyatta National Hospital, Peter Mwatha, in-charge of parasitology

laboratory, Kenyatta National Hospital in collaboration with Felista Muthini, in-charge of parasitology laboratory, University of Nairobi.

They said it is possible and agreed but they advised me to seek for an authorization from your office. Sir, I am therefore, making a kind request for the above mentioned permit. Attached here, please find a copy of my research proposal, observations, suggestions from KNH-UoN Ethics and Research Committee and methodologies. Thanking you in advance for your kind consideration,

Yours faithfully

Theresia Ndunda

APPENDIX IX: Approval to use Specimens from Blood Transfusion Unit Bank at KNH.



KENYATTA NATIONAL HOSPITAL
P.O. Box 20723, 00200, KNH
NAIROBI
Tel: 2726300- 9
Fax: 2725272
Telegrams: "MEDSUP" Nairobi

KNH/DLM/60/Vol.I/(145)

Date: 29th July, 2016

Teresia Ndunda
Technical University of Kenya
P. O. Box 52428-00200
NAIROBI

Dear Madam,

RE: PERMISSION TO USE SPECIMENS FROM BLOOD TRANSFUSION UNIT BANK AT KNH

Reference is made to your letter for the permission to use specimens from Blood Transfusion Unit on your Research entitled "Determination of Risks of Transmitted Malaria Parasites among Blood Donations made at KNH blood Transfusion Unit"

The department of Laboratory Medicine has reviewed the methodology in your proposal to the Ethics and Research Committee. We have no objection in your research study being conducted as it falls within our core mandate.

You will therefore be required to make payments for the available test, Thick blood film test. The payments will have to be paid through the Finance department code No.12093 blood slide for Mps. This payment will be paid in advance for the whole of your sample size of 155. As a general guide the test is charged at 100/= and therefore you will have to pay Ksh.15,500/=. You will not be required to pay for Rapid diagnostic and the quantitative blood test whose reagents are not available in the Hospital.

You are advised further to get in touch with the in-charge Blood Transfusion Unit for advice in the logistics. The In-charge of Blood Transfusion will also identify the persons in the section who you can appoint as your assistants to coordinate the technical procedures on behalf of the Hospital. The assistants will be responsible for the release of your results and their dispatch to you through your supervisor, Dr. Kibet Shikuku of the Dept. of Haematology, University of Nairobi.

Finally, please note that you will have to present the payment receipt for the test and a Registration Certificate from the Department of Research and Programs to our department before you commence the study.

Dr. A.K.Gachii
Ag. AD LABORATORY MEDICINE

Cc. The HOD Research & Programs-KNH
The SAD - Finance -KNH
Dr. Kibet Shikuku - UON
In-Charge - BTU

Our Vision: To Be A World Class Centre in The Provision Of Innovative And Specialized Medical Laboratory Services.

ISO 9001:2008 CERTIFIED



APPENDIX X: Ethical Approval



UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
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Ref: KNH-ERC/ Mod&SAE/232

22nd September 2017

Theresia M. Ndunda
Reg. No. TM300-1272/2013
Dept. of Medical Laboratory Science
JKUAT

Dear Theresia

Re: Request for approval of modifications – study titled 'Prevalence of transfusion transmitted malaria parasites among blood donors and evaluation of two testing methodologies at Kenyatta National Hospital (P116/02/2016)

Refer to your communication of September 6, 2017.

Upon review of the requested modifications, the KNH-UoN ERC hereby approves the change of study title from 'Determination of risks of transfusion transmitted malaria parasite among blood donors attending Kenyatta National Hospital, Blood Transfusion Unit, Kenya' to '**Prevalence of transfusion transmitted malaria parasites among blood donors and evaluation of two testing methodologies at Kenyatta National Hospital, Kenya**'.

Yours sincerely,

A handwritten signature in black ink, appearing to read "M.L. Chindia".

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

c.c. The Principal, College of Health Sciences, UoN
The Director CS, KNH
The Chair, KNH-UoN ERC

Protect to discover



Evaluation of Two Transfusion Transmitted Malaria Screening Methods at Kenyatta National Hospital, Kenya

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ABSTRACT

Malaria is a disease responsible for morbidity and mortality in Sub-Saharan Africa. The primary mode of transmission is through the bite of an infected female Anopheles mosquito. There is transfusion transmitted malaria following blood transfusion in medical facilities. **Specific objectives:** This study was designed to determine the prevalence of malaria parasite among voluntary blood donors and to evaluate the performance characteristics of Quantitative buffy coat and Rapid diagnostic technique using CareStart™ malaria cassette from AccessBio manufacture were compared. Microscopy was used as the gold standard. **Design:** Cross sectional descriptive study. **Subjects:** Voluntary blood donors. **Results:** A total of 155 blood donors were recruited in this study and blood samples were screened for malaria parasites. The prevalence of malaria parasite using microscopy, rapid diagnostic test and quantitative buffy coat was 5/155 (3.2%), 8/155 (5.2%) and 6/155 (3.9%). The sensitivity of rapid diagnostic test and quantitative buffy coat was 80% and 100% while the specificity was 99% and 97%. The positive predictive values for rapid diagnostic test and quantitative buffy coat were 50% and 83% while the negative predictive values were 99% and 100%. **Conclusion:** This study concluded that some blood donors have malaria infection since prevalence of malaria parasite in blood donors was 3.2%. Quantitative buffy coat technique was more sensitive and with higher specificity. **Recommendations:** This study recommends that blood and its products for transfusion should be screened for malaria parasites. The quantitative buffy coat technique should be used for malaria diagnosis.

Keywords: transfusion transmitted malaria, quantitative buffy coat, rapid diagnostic technique

1. INTRODUCTION

Malaria is a devastating mosquito borne illness that is predominantly prevalent in the tropical and subtropical regions of the world. Malaria is mainly transmitted through the bite of an infected female Anopheles mosquito. Transmission can also occur through transfusion of infected blood and its products. Epidemiological studies in malaria-endemic areas have reported a prevalence of malaria among potential blood donors to range between 1% and >50% (1). The risk of Plasmodium transmission through blood transfusion is accounted for by the persistence of malaria parasites in the blood. It has been estimated that nearly half of the world's population is at risk of contracting malaria with Africa

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being the most malaria risky area (2). Malaria is one of the world's deadliest diseases affecting people particularly in tropical and sub-tropical regions of the world with 300 to 500 million cases and 2 to 3 million deaths per year (3). Kenya being an endemic region, transfusion transmitted malaria may result with relatively higher mortality, particularly among the vulnerable sections of the society like pregnant women, infants, splenectomized and immune compromised patients as a result of tardy diagnosis and treatment (4). A recent study demonstrated a relatively high likelihood of transfusion transmitted malaria in sub-Saharan African countries, illustrated by a median prevalence of malaria and determined by the evaluation of thick smears of 10.2% (range 0.7% in Kenya to 55% in Nigeria) in donor blood samples (5). Blood and its products for transfusion are not frequently screened for malaria parasites thus there is need for effective screening tool for transfusion transmitted malaria.

2. METHODS

This was a descriptive cross sectional study. An informed written, and voluntary consent was obtained from blood donors before collection of samples for screening. After the participants had given their written consent a malaria questionnaire was administered to collect the required information. Following completion of the questionnaire, blood samples were collected and processed. Specifically, a two milliliters blood sample was collected from each donor through the cubital vein. This sample was then transferred into a 5ml EDTA tube. The screening tests were conducted by the principal investigator and confirmed by an assistant registered medical laboratory technologist. Microscopy was used as the gold standard.

Test methods

Microscopic technique: Thick peripheral blood smears were prepared from each donor on microscope slides and stained using the Giemsa method. The smears were examined under oil immersion objective using ordinary microscope for the presence or absence of parasites.

Quantitative buffy coat technique: A tube was filled with 50 microliters of blood from the donor. A stopper and float were placed at the end of the tube and the tube was then centrifuged using a microhaematocrit centrifuge at 12,000 rpm for 5 minutes. The centrifuged tube was placed in a tube holder and examined using $\times 60$ objective of a fluorescent microscope. Results were reported in terms of presence or absence of parasites.

Rapid diagnostic technique: This technique was performed using Carestart TM malaria cassettes from AccessBio. Two milliliters of donor's blood were collected in a 5ml lavender tube containing EDTA anticoagulant. Five microliters of each blood sample was aspirated using a pipette which was then added on the sample well labelled S on the malaria cassette. Two drops of buffer solution were then added on the buffer well labelled A. Test results were read after 20 minutes.

Data analysis: Data collected was analyzed using Kappa statistic to determine if there was a level of agreement between quantitative buffy coat and rapid diagnostic techniques against microscopy.

3. RESULTS

Prevalence of malaria parasite among blood donors using malaria screening techniques.

Table 1: Prevalence of malaria parasite among blood donors.

Technique	Blood donor samples tested	
	Positive for malaria parasites	Prevalence (%)
Microscopy	5	3.2
Rapid Diagnostic Test	8	5.2
Quantitative Buffy Coat Technique	6	3.9

The prevalence of malaria parasite among blood donors was determined using rapid diagnostic

technique and quantitative buffy coat and they were compared against microscopy. Microscopy, Rapid

diagnostic technique and quantitative buffy coat reported prevalence of 5/155 (3.2%), 8/155 (5.2 %) and 6/155 (3.9 %) respectively as shown on the table 1 above.

Evaluating the performance characteristics of quantitative buffy coat and rapid diagnostic techniques against microscopic technique.

Table 2: Evaluating the performance characteristics of two malaria screening techniques. Microscopic technique was used as the gold standard.

Malaria Screening Technique	Performance characteristics			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Microscopy	100	100	100	100
Quantitative buffy coat	100	99	83	100
Rapid diagnostic test	80	97	50	99

The sensitivity of microscopy, rapid diagnostic test and quantitative buffy coat was, 80% and 100% respectively. The specificity of these malaria infection screening tools was reported to be 99% and 97% respectively. The positive predictive values for rapid diagnostic test and quantitative buffy coat was reported to be 50 % and 83% respectively. The

negative predictive values reported for the screening techniques was 99% and 100 % respectively.

Determining level of agreement of quantitative buffy coat and rapid diagnostic techniques against the gold standard.

Table 3: Level of agreement between rapid diagnostic test and microscopy technique in detection of parasites.

		Microscopy Technique - Gold Standard		
		Positive	Negative	Total
RAPID DIAGNOSTIC TEST Technique	Positive	4	4	8
	Negative	1	146	147
	Total	5	150	155

The level of agreement between rapid diagnostic technique and microscopy was determined using Kappa statistic. A 2x2 contingency table was used to

tabulate the results as shown in the table 3 above. The kappa value resulted to 0.63.

Table 4: Level of agreement between quantitative buffy coat and microscopy technique in detection of parasites.

		Microscopy Technique - Gold Standard		
		Positive	Negative	Total
QUANTITATIVE BUFFY COAT Technique	Positive	5	1	6
	Negative	0	149	149
	Total	5	150	155

The level of agreement between quantitative buffy coat and microscopy was determined using Kappa statistic. A 2x2 contingency table was used to tabulate the results as shown in the table 4 above. Microscopy which was used as the gold standard in malaria infection screening reported 5 true positives (TP),150 true negatives (TN),0 false positives (FP) and 0 false

negative (FN) cases. Rapid diagnostic test reported 4 true positives (TP),146 true negatives (TN),4 false positives (FP) and 1 false negative (FN) cases. Quantitative buffy coat technique reported 5 true positives (TP), 149 true negatives (TN),1 false positive (FP) and 0 false negative (FN) cases as tabulated in table 5.

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Table 5: Predictive values of blood donor samples analyzed using the two techniques against microscopy technique.

Technique	TEST RESULTS			
	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)
Microscopy	5	150	0	0
Rapid Diagnostic Test	4	146	4	1
Quantitative Buffy Coat	5	149	1	0

4. DISCUSSION

The transmission of malaria infection through blood transfusion is one of the oldest recorded incidents of transfusion transmitted infections in transfusion facilities due to lack of screening. Malaria transmissions associated with blood transfusion in malaria prone zones has been on the rise. This study has confirmed that blood donors that are seen at Kenyatta National Hospital have a prevalence of 3.2%. Based on observations from malaria screening questionnaire directed to asymptotology of blood donors; this study has showed that voluntary blood donors contribute to the risk of transfusion transmitted malaria in low and high prevalence zones. While Nairobi is classified as low malaria endemic zone transmission of malaria through blood transfusion is still present. According to a study by Kitchen AD, Chiodini PL, and Tayou T.C, et al., (6,7) which showed that transmission of malaria infection by blood transfusion is a possible cause of malaria infections among blood donors and it agrees with this study. Other similar studies carried out including a study carried out in Abakaliki Metropolis, Epedi et al., (8) obtained a prevalence of 40.9% among blood donors which was high contrary to this study. The difference in those studies and this study could have been caused by differences in sample size used, endemicity, period during which the data was collected and the technique used for diagnosis. A lower prevalence of 1% was reported in Ethiopia among blood donors by Sarr A, et al (9). The prevalence reported in this study was comparable to other studies that were done in Ghana (10%) Owusu-Ofori, A, Gadzo D, Bates I (10) and Yaounde 6.5%) Noubouossie, D, Tagny CT, Same-Ekobo AMbanya D. (11).

In this study, the prevalence of malaria infection among blood donors was found to be 3.2% by microscopy, quantitative buffy coat 3.9 % while the rapid diagnostic test gave a prevalence of 5.2 %. quantitative buffy coat technique results compared well with microscopy because it picked all positives

picked by the gold standard. Rapid diagnostic test yielded higher prevalence values above the gold standard because it picked more false positive values than microscopy. rapid diagnostic test was found to detect more false positives than quantitative buffy coat and microscopy. This means that rapid diagnostic test is more prone to false positives than the gold standard. This could have been attributed by confounders such drug resistance, persistence of antigens due to sequestration, incomplete malaria treatment, cross reaction with autoantibodies such as rheumatoid factor and presence of histidine rich protein 2 antigen of plasmodium parasite in the blood of the donor gene deletion or mutation of Histidine Rich Protein-2 as postulated by Wellems et al., (12) and this agreed with a study that was done (13). The rapid diagnostic test was less accurate in determination of malaria prevalence compared to the quantitative buffy coat.

Rapid diagnostic test and quantitative buffy coat showed a sensitivity of 80% and 100% respectively. The sensitivity of quantitative buffy coat compared well with the gold standard. This meets the WHO recommended cut off of 95% (14). In this study, the sensitivity of the two malaria screening techniques indicates that quantitative buffy coat technique was highly sensitive in detection of TTM while rapid diagnostic test was less sensitive. A similar study which was conducted in Cameroon by Kwenti TE et al., 2017(15) rapid diagnostic test showed a sensitivity of (88.0%). The rapid diagnostic test produced lower sensitivity values compared to the gold standard and fell below the recommended values of over 95% as it is required by WHO.

This study depicted the specificity of rapid diagnostic test and quantitative buffy coat as 97% and 100% respectively. The same study done by B.Latha et al 2016 in India reported rapid diagnostic test and quantitative buffy coat to have specificities of 100% and 100 % respectively. The specificity of quantitative buffy coat which was 100% from that study agreed strongly with the findings from this study on same technique. The specificity of rapid diagnostic test of

97% agreed favorably which the study which was done in India. The specificity of rapid diagnostic test compared favorably with microscopy and the specificity of quantitative buffy coat compared well because it picked all positives values picked by the gold standard. In this study, the positive predictive value for rapid diagnostic test and the quantitative buffy coat was 83% and 50% while the negative predictive values were 100% and 99% respectively. The level of agreement of quantitative buffy coat and rapid diagnostic techniques against microscopy was determined using Kappa statistic. The study found that the Kappa value for quantitative buffy coat compared to microscopic technique was 0.86. This implied that there was almost perfect agreement between quantitative buffy coat and microscopy technique. The study reported a kappa value of 0.63 between the rapid

diagnostic technique and the microscopy. This implies that there was substantial agreement between the gold standard and the rapid diagnostic test. The study findings agreed with the conclusion arrived (16) that microscopy and quantitative buffy coat techniques are good tools of malaria screening.

ACKNOWLEDGMENTS

May I take this opportunity to express my sincere gratitude to my supervisors Dr. Amos Mbugua, Dr. Kibet Shikuku and Dr. Naomi Waiganjo for their valuable guidance, constant supervision and dedication making my research work successful. We take this opportunity and appreciate Aglean Chelimo and Perpetual Asuko for their support during donors' recruitment process. Special thanks goes to Mrs. Felistas Sino, who assisted in confirming microscopic, quantitative and rapid diagnostic results for malaria diagnosis. We would also like to thank Mr. Stephen Musimba for data analysis and to all our research participants.

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