DETERMINING THE EFFICIENCY OF MALARIA RAPID DIAGNOSTIC TEST TO DIAGNOSE POST TRANSFUSION MALARIA IN CHILDREN UNDER FIVE YEARS OLD AT KOMFO ANOKYE TEACHING HOSPITAL

By

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DECLARATION

I hereby declare that, this thesis submission is my own work towards the MSc in Clinical Microbiology and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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DEDICATION

I dedicate this work to my dearest dad, Mr. Sékou Bouaré.

ACKNOWLEDGEMENT

To the Almighty GOD be the glory WHO has seen me through from the beginning to the end of this work. Blessed be Your Name, Exalted be Your Majesty.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACT: Artemisinin-based Combination Therapy.

AQ: Amodiaquine.

AL: Artemether Lumefantrine.

AS: Artesunate.

CI: Confidence Interval.

DNA: Deoxyribonucleic Acid.

EDTA: Ethylene Diamine Tetra-Acetate.

HBV: Hepatitis B Virus.

HCV: Hepatitis C Virus.

HIV 1 & 2: Human Immunodeficiency Virus 1 & 2.

KATH: Komfo Anokye Teaching Hospital.

KCCR: Kumasi Centre for Collaborative Research.

KNUST: Kwame Nkrumah University of Science and Technology.

MBU: Mother and Baby Unit.

NPV: Negative Predictive Value.

OPD: Outpatients Department.

PCR: Polymerase Chain Reaction.

PEU: Paediatric Emergency Department.

Pfhrp2: *Plasmodium falciparum* histidine rich protein 2.

PICU: Paediatric Intensive Care Unit.

PLDH: Plasmodial Lactate Dehydrogenase.

PPV: Positive Predictive Value.

QN: Quinine.

RBCs: Red Blood Cells.

RDT: Rapid Diagnostic Test.

RNA: Ribonucleic Acid.

SENS: Sensitivity.

SMS: School of Medical Sciences.

SP: Sulphadoxine Pyrimethamine.

SPES: Specificity.

TBE: Tris-Borate with EDTA.

TMU: Transfusion Medicine Department.

TTBI: Transfusion Transmitted Bacterial Infection.

TTI: Transfusion Transmitted Infection.

TTM: Transfusion Transmitted Malaria.

WBCs: White Blood Cells.

WHO: World Health Organization.

ZIKV: Zika Virus.

ABSTRACT

Malaria associated with blood transfusion in malaria-endemic countries has until recently been a neglected area. Malaria prophylaxis has however been consistently used for the management of transfusion transmitted malaria (TTM). Current recommendations from World Health Organization (WHO) state that, the diagnosis of malaria must be confirmed before initiation of therapy. It is therefore important to establish which of the current routine test methods, is most appropriate for use. Malaria RDTs are now supplementing microscopy in the diagnosis of malaria in many endemic countries. However their efficiency as a screening tool for the detection of malaria in donors or in transfusion recipients has not been well evaluated.

The aim of this study was to determine the efficiency of malaria RDT in the detection of post transfusion malaria in children under five years old at Komfo Anokye Teaching Hospital (KATH), Kumasi.

The study took place between November 2014 and April 2015. Blood samples from 179 patients and their corresponding donors were tested for *Plasmodium* parasitaemia using RDT and microscopy. The recipients' blood samples were obtained within 48hours after transfusion for malaria diagnosis. To determine the incidence of post transfusion malaria, 32 patients were purposefully selected. Their pre-transfusion, post transfusion and donor blood samples were tested for malaria using PCR.

Majority (52%) of the study population was between the ages of 0 - < 1 year. Prevalence of malaria in blood donors was 27% by RDT and 7% by microscopy. Prevalence at pre transfusion, was 34% by RDT and 13% by microscopy. Prevalence at post transfusion was 59% by RDT and 4% by microscopy. Incidence of post transfusion malaria by PCR was 57%. The sensitivity of RDT in detecting malaria at post transfusion was 100%. Efficiency of malaria RDT as a tool for malaria diagnosis at post transfusion, among children under five years old is good. Clinicians should consider implementing the use of malaria RDT at post transfusion.

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CHAPTER 1: INTRODUCTION

1.1 Study background

Malaria is one of the major causes of child death in Africa (Elphinstone *et al*, 2014). According to the latest estimates of World Health Organization (WHO), 198 million cases of malaria occurred globally and the disease led to 584 000 deaths. The burden is heaviest in Africa, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, who account for 78% of all deaths (WHO, 2014).

In Ghana, malaria is a burden among children under five years old (Nyarko & Cobblah, 2014). An estimated 3.1 to 3.5 million cases of clinical malaria are reported annually in public health facilities, of which 20,000 cases were children under five years old (Buabeng *et al*, 2007). Malaria is one of the leading causes of childhood death in Ghana (Nyarko & Cobblah, 2014) with blood transfusion being one of the major sources of malaria transmission (Owusu-Ofori, 2012).

Malaria occurring after transfusion could simply be described as post transfusion malaria. Post transfusion malaria could be due to parasites acquired by mosquitos' bites or could be acquired from the transfused blood. When post transfusion malaria is due to parasites that were in the donor blood, it is described as transfusion transmitted malaria (TTM). Evidence exists of TTM that has occurred both in Ghana and worldwide (Owusu-Ofori, 2012; O'Brien *et al*, 2015).

In Sub Saharan Africa the demand for blood transfusion is high especially among children under five years old and pregnant women (Austin *et al*, 2014; Erhabor & Adias, 2011). Blood donors are not screened for malaria in Sub Saharan Africa and Ghana, despite being recommended by WHO (Mark & Shauna, 2005). In order to prevent post transfusion malaria, anti-malarial treatment is sometimes given to recipients after transfusion, in Sub Saharan Africa (Jobert *et al*, 2013; Tayou *et al*, 2007). Post transfusion malaria can have an impact on the health of non-immune children with complications ranging from severe anaemia to death (Jobert *et al*, 2013).

Several reasons have been advanced for why, it is difficult to routinely screen blood donors for malaria, in Ghana and the rest of Sub Saharan Africa. Some of the reasons are the non-availability of a sensitive malaria screening method by blood bank services and the severe blood shortages which are already widespread and may be exacerbated by rejecting blood that contains malaria parasites, as asymptomatic carriers are common in endemic areas (Bates *et al*, 2011).

The available routine malaria screening methods in malaria endemic areas are light microscopy and malaria RDTs.

Malaria RDTs are immune chromatographic tests made to detect the antigens of malaria parasites (Kátia *et al*, 2014). They could be specific to one species using a monoclonal antibody or specific to more than one species using a polyclonal antibody (Weekley & Smith, 2013). The use of malaria RDTs is widespread nowadays and it is, not time consuming, does not require skilled labour and it is cost effective (Joel & Dean, 2013; Chou *et al*, 2012). Malaria RDTs could however give false positive and false negative results (Kakkilaya, 1990).

Microscopy is the gold standard for malaria diagnosis (Li *et al*, 2013). It helps to detect the presence of malaria parasites from peripheral blood smears. Hence it requires a skilled person and a very well stained slide. However it is time consuming, tedious and cannot be used at most peripheral areas where there is a lack of electricity (Mali *et al*, 2012). The sensitivity of microscopy decreases when the parasitaemia is low (Johnston *et al*, 2006).

Up to now, there is no evidence based guidance to indicate which malaria screening methods are effective for use by transfusion services (Lund *et al*, 2013).

More consideration must then be given to post transfusion malaria especially among children under five years old in order to increase the continuous management of childhood illnesses and death (Nyarko & Cobblah, 2014; Bhutta *et al*, 2010).

1.2 Problem statement

Screening of malaria is currently not done in blood donors and recipients in KATH. Despite this, anti-malarial drugs are being prescribed indiscriminately to transfused recipients.

Children under the age of five are part of the majority of patients who received blood transfusion at KATH. They are one of the highest victims of malaria in Ghana through various ways of transmission, including blood transfusion. Anti-malarial treatment is sometimes given to recipients after blood transfusion without confirmation of malaria infection. This is contrary to WHO recommendations which require laboratory confirmation before initiation of treatment. The lack of an ideal test method to detect the low parasitaemia may have contributed to the lack of compliance hence inappropriate treatment. An ideal test usually refer to a diagnostic technique that is readily available, affordable, easy to be used and most accurate.

The inappropriate treatment of malaria could lead, to the development and spread of malaria drugs resistances and the inability to control malaria.

1.3 Justification of the study

A lot of studies have been done on post transfusion malaria in endemic and non-endemic areas. There is however not much published evidence on the evaluation of malaria RDT in the diagnosis of post transfusion malaria. The data from this study could provide a perspective on the diagnosis of post transfusion malaria in children under five years old. Microscopy and malaria RDT are the malaria diagnosis tools available in KATH. Microscopy being the gold standard for malaria diagnosis has been the method that is frequently used in KATH.

In KATH there is no data on post transfusion malaria diagnosis. If the efficiency of malaria RDT to diagnose post transfusion malaria is shown to be acceptable, physicians will now be able to confirm malaria in transfused recipients before treatment. This could lead to the prompt treatment of recipients, improvement in compliance and the reduction of malaria drugs resistances.

1.4 General objective of the study

To determine the efficiency of malaria rapid diagnostic test (RDT) as a detection tool for post transfusion malaria in children under 5 years old.

1.5 The specific objectives of the study were:

1- To determine the prevalence of *Plasmodium* parasitaemia in blood donors.

2- To determine the prevalence of *Plasmodium* parasitaemia in recipients prior to transfusion.

3- To determine the incidence of post transfusion malaria.

4- To establish the sensitivity of malaria rapid diagnostic test (RDT) to detect post transfusion malaria.

CHAPTER 2: LITERATURE REVIEW

2.1 Global overview about malaria

Malaria is an important and complex parasitic infectious febrile disease with a worldwide distribution (Elphinstone *et al*, 2014; Austin *et al*, 2014). Its epidemiology differs from area to area (Reyburn *et al*, 2007). In malaria endemic areas, such as Sub Saharan Africa, the disease has a high mortality (Greenwood, 2010; Greenwood & Mutabingwa, 2002) especially among children under five years old and pregnant women.

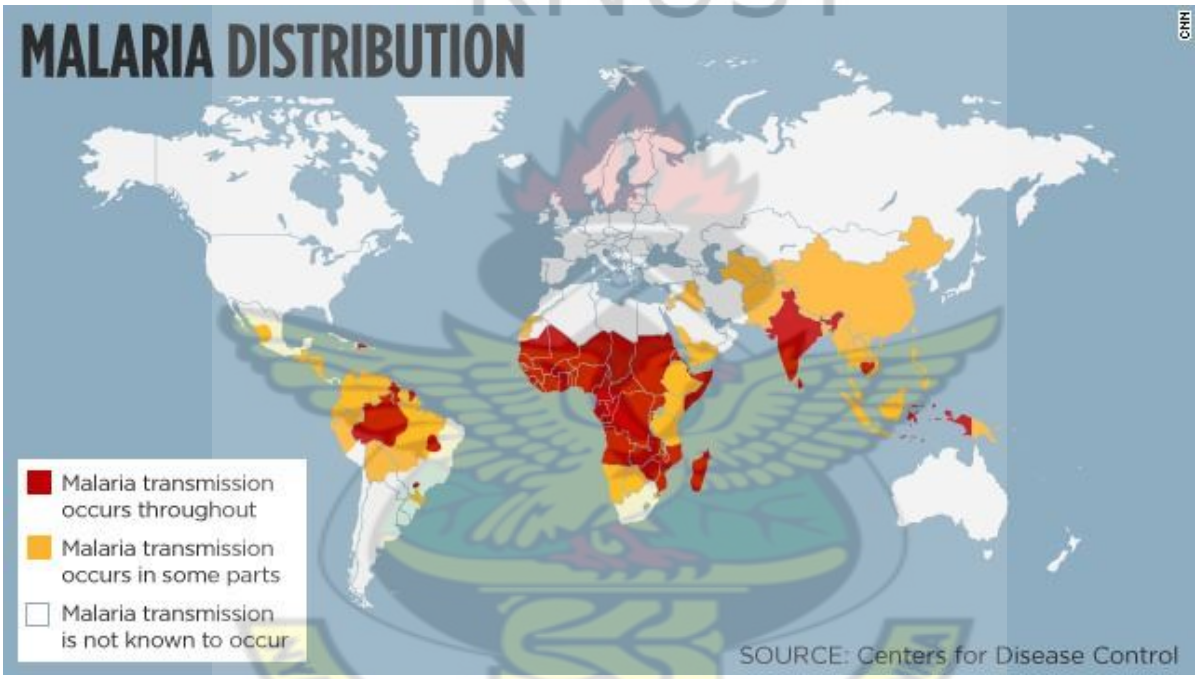

Figure 2.1: Distribution of malaria in the world (2010).

2.1.1 Malaria parasites

Malaria is caused by protozoan parasites called *Plasmodia*, belonging to the parasitic phylum *Apicomplexa* (Keating *et al*, 2014). More than 200 species of the genus *Plasmodium* exist. They are parasitic to reptiles, birds, and mammals. Four *Plasmodium* species are well known to cause human malaria. They are, *P. falciparum, P. vivax, P. ovale, and P. malariae.* (Li *et al*, 2013; Freedman, 2008; Tagny *et al*, 2008). In addition, *P. knowlesi*, a malaria parasite of monkeys,

has been recorded as a cause of human's infection in many countries of Southeast Asia (Autino *et al*, 2012; Cox-Singh *et al*, 2008). Very rare cases of malaria have been reported due to other species such as *Plasmodium brasilianum, Plasmodium cynomolgi, Plasmodium inui,* and *Plasmodium eylesi* (Kakkilaya, 1990).

2.1.2 Importance of malaria parasites

The severity of infection caused by malaria parasites depends on species specific and host immunity. *P. falciparum* differs from the other species because, it is responsible of fatal malaria (Prugnolle *et al*, 2010). It infects erythrocytes of all ages and hence capable of producing high parasitaemia (Andrej *et al*, 2003).

However *P. vivax* and *P. ovale* infect youngest erythrocytes whereas *P. malariae* infects oldest erythrocytes, reducing their capacity to produce high parasitaemia (Tembo, 2013).

2.2 Epidemiology of malaria

2.2.1 Worldwide epidemiology

Malaria is a worry to human health worldwide. There are over 250 million cases of malaria each year, and nearly 1 million deaths (Kelly *et al*, 2015). About 106 countries and territories (Weekley & Smith, 2013) are at risk of malaria disease. In non-malaria endemic countries, the presence of malaria is predominantly due to imported malaria by travellers from malaria endemic countries (João *et al*, 2014; Kitchen *et al*, 2005).

2.2.2 Malaria in Sub Saharan Africa

Malaria is one of the major public health problem in Sub Saharan Africa causing high morbidity and mortality (Keating *et al*, 2014). A study showed that, up to 300-500 million infections and 1 (one) million death among children under 5 years old and pregnant women are recorded annually in Sub Saharan Africa (Eisele *et al*, 2012). Most children in malaria endemic areas

experience their first malaria infection during their first two years (Jobert *et al*, 2013). At that moment they have not yet acquired adequate immunity to overcome a little density of malaria parasites. That makes the early years of life the phase with highest risk. Consequently 90% of all malaria deaths in Africa occur in young children. According to WHO reports, one African child dies every minute from malaria disease (Hendriksen *et al*, 2012). The diversity of malaria parasites and the favourable environment could be a contributing factor to the difficulties of malaria control in Sub Saharan Africa and Ghana.

2.2.3 Malaria in Ghana

Malaria remains one of the major causes of poverty, low productivity, illness and death in Ghana, particularly among children under the age of five and pregnant women (Asante *et al*, 2010; Freedman, 2008; Sachs & Malaney, 2002). Malaria is hyper-endemic in Ghana, accounting for 44% of outpatient attendance, 13% of all hospital deaths, and 22% of mortality among children less than five years of age. (Abdul-Aziz et al, 2012).

The prevalence of malaria parasitaemia among children living in Kumasi varies from 79% to 85%. The available data suggest that less than one third of malaria diagnosis are confirmed with laboratory tests (President's Malaria Initiative, 2013b). The available malaria diagnosis tools at the Komfo Anokye Teaching Hospital (KATH) of Kumasi are light microscopy and malaria RDT.

Malaria is significant in Ghana because most malaria cases are managed at the household level (Buateng et al, 2007) prior to hospital attendance, followed by self-medication and inappropriate use of anti-malarial drugs without any precise diagnosis which could have contributed to the spread of anti-malarial drugs resistances (Alam *et al*, 2011).

2.3 Malaria policy in Ghana

A report by the National Malaria Control Programme (2014) showed that, since 2013 Ghana was pursuing the 3T policy (Test, Treat and Track). But the report showed that not all suspected malaria cases were tested under the 3T policy. This means, till now malaria drugs are given to patients without malaria diagnosis. This goes against WHO recommendations which is test before initiating treatment (Bastiaens *et al*, 2014). This practice could have contributed to the development and spread of malaria drugs resistances (Keating *et al*, 2014). In order to diminish cases of malaria drugs resistances, to provide prompt, safe, effective and appropriate anti-malaria treatment to the entire population of Ghana the Ministry of health (2009) had established a policy guidelines for malaria treatment (**Table 2.1**).

Anti-malarial drugs	Cases
Artesunate-Amodiaquine combination	Management of uncomplicated malaria
Quinine Artesunate-Amodiaquine Artemeter-Lumenfantrine	Management of uncomplicated malaria in pregnancy
Artesunate-Amodiaquine	Home management of uncomplicated malaria
Quinine Artemisinin-based Combination Therapy (under prescription) Artesunate-Amodiaquine Artemether-Lumenfantrine	Treatment failure
Quinine Intravenous Artemether	Complicated malaria

Table 2.1: Malaria treatment guidelines in Ghana (Ministry of health, 2009).

2.4 Transmission of malaria

All the four species are transmitted to human by the bite of an infective female *Anopheles* mosquito (Crompton *et al,* 2014). However humans can also be infected through other means which are: congenital transmission (mother to child), organ transplantation (especially liver), needle sharing especially among intravenous drugs users and blood transfusion (Weekley & Smith, 2013).

2.5 Clinical presentation of malaria

After a period of pre-erythrocytic development in the liver, which is the first stage after a bite by a mosquito, the blood stage follows, which causes the disease malaria (Prugnolle *et al*, 2010). The clinical manifestations of malaria vary with geography, epidemiology, immunity, and age. In malaria endemic areas, the group of highest risk includes children under five years old who can develop severe illness, and pregnant women, who are at risk for anaemia and delivering of low birth weight new-borns. In malaria endemic areas older children and adults develop partial immunity after repeated infections and are at relatively low risk for severe disease (Kochar *et al,* 2010; Marc *et al,* 2013). Infection with malaria parasites may result in a wide variety of symptoms, ranging from absence or mild symptoms to severe disease and even death (Tembo, 2013). However the symptoms may develop as early as 7 days after initial exposure.

All the clinical symptoms associated with malaria are caused by the asexual erythrocytic stage parasites. That can be acquired directly after receiving a transfusion of *Plasmodium* infected blood. When the parasite develops in the erythrocyte, numerous substances such as haemozoin pigment and other toxic factors, accumulate in the infected RBCs. These are released into the bloodstream when the infected RBCs lyse and release invasive merozoites. The haemozoin and other toxic factors such as glucose phosphate isomerase stimulate macrophages and other cells to produce cytokines and other soluble factors which act to produce fever. This stimulation is also characterized by flu like symptoms, chills, headache, myalgia, and malaise (Mackintosh *et al,* 2004; Orla & Imogen, 2014).

Uncomplicated malaria may be associated with anaemia and jaundice. In severe malaria mostly cause by *P. falciparum*, seizures, mental confusion, kidney failure, acute respiratory distress syndrome, coma, and death may occur (Asante *et al*, 2010).

Both *P. vivax* and *P. ovale* can become dormant in the liver, forming hypnozoites that may reappear months to years after the initial infection to cause disease. Recipients with *P. vivax*

infection commonly present with paroxysmal fevers, chills, headaches, and myalgia. *P. malariae* and *P. ovale* generally cause fever but not a toxic appearance (William & Philip, 2003).

2.6 Diagnosis of malaria

Diagnosis of malaria involves, identification of malaria parasites or their antigens/products in a specimen. The diagnosis of malaria is routinely confirmed by blood tests and can be divided into microscopic and non-microscopic tests (Akotet *et al*, 2014). Tests for malaria parasites detection include thick and thin blood smears for microscopy, fluorescent staining techniques, RDTs for circulating malarial antigens, PCR for detection of malarial deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and serological tests detecting antibody (Chanda *et al*, 2011; Micheal, 2013). Serological antibody testing does not indicate parasitaemia because antibody can remain elevated in the blood up to years after infection; it is mostly used in non-malaria endemic areas (Choudhury, 2010).

2.6.1 Diagnosis of malaria using microscopy

Microscopic test involves staining and direct visualization of parasites under the microscope (Tek *et al*, 2009). Light microscopy remains the gold standard for malaria diagnosis (Acheampong *et al*, 2011).

2.6.1.1 Peripheral smear for malaria parasites detection

The smear can be prepared from blood collected by venepuncture and or finger prick. Peripheral smear involves collection of blood, thick and/or thin smear (s) preparation, its staining with Romanowsky stains (composed of Giemsa, Jenner, Wright, Field, and Leishman stain, with Giemsa stain being the most used for malaria microscopy) (Endeshaw, *et al*, 2008) and examination of the smear (s) under microscope for the presence of malaria parasites. The efficacy of the test requires great care at each step (Tek, *et al*, 2009).

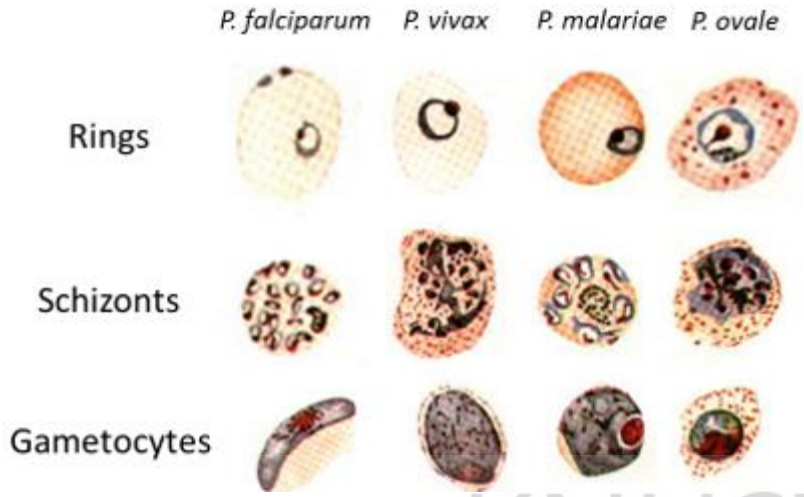

Figure 2.2: Malaria parasites' species/stages in RBCs (Kakkilaya, 1990).

2.6.1.2 Targets of microscopy for malaria parasites detection

In thick blood smear, the RBCs are lysed. By so doing, the parasites are released in the smear. The thick smear is used to detect the presence or absence of malaria parasites. Malaria parasites are counted in relation to a predetermined number of WBCs and an average of 8000/µl is taken as standard. The thin smear is one layer of blood in which malaria parasites could be found in RBCs. Depending on the species causing infection the RBCs are deformed or not. In thin film the parasitaemia is determined by the number of infected RBCs (and not number of parasites) divided by 1000 uninfected RBCs. This method estimates the percentage of red blood cells infected with malarial parasites (Alves-Junior *et al*, 2014). The smears are scanned carefully, one 'row' at a time. The thin smear is used to determine the species causing infection as well as the level of parasitaemia (Acheampong *et al*, 2011; Mali *et al*, 2012).

2.6.1.3 Problems with malaria microscopy

The exacting needs of the blood smear examination are often not met in certain remote and poor parts of the world. Sometimes no parasite can be found in peripheral blood smear from patients with malaria, even in severe infection (Mbakilwa *et al*, 2012). Besides difficulties in

maintaining the required technical skills and resultant misdiagnosis due to poor training are some of the deficiencies with the blood smear examination (Mukadi *et al*, 2011).

2.6.2 Diagnosis of malaria using malaria RDT

Malaria RDTs are immune-chromatographic tests, for the detection of malaria parasites antigens (Bahadur *et al*, 2010) using either monoclonal or polyclonal antibodies (Endeshaw *et al*, 2008). There are different test formats like the dipstick, strip, card, pad, well, or cassette. The test procedure varies between test kits (Mudanyali *et al*, 2012). In general, the blood specimen (2 to 50µL) is either a finger-prick blood specimen, anti-coagulated blood, or plasma, and it is mixed with a buffer solution that contains a haemolysing compound and a specific antibody that is labelled with a visually detectable marker (Shekalaghe *et al*, 2013). If the target antigen is present in the blood, a labelled antigen/antibody complex is formed and it migrates up the test strip to be captured by the pre-deposited capture antibody specific against the antigen and against the labelled antibody and permit visualization of any coloured line formed by the immobilized antigen-antibody complex (Choudhury, 2010).

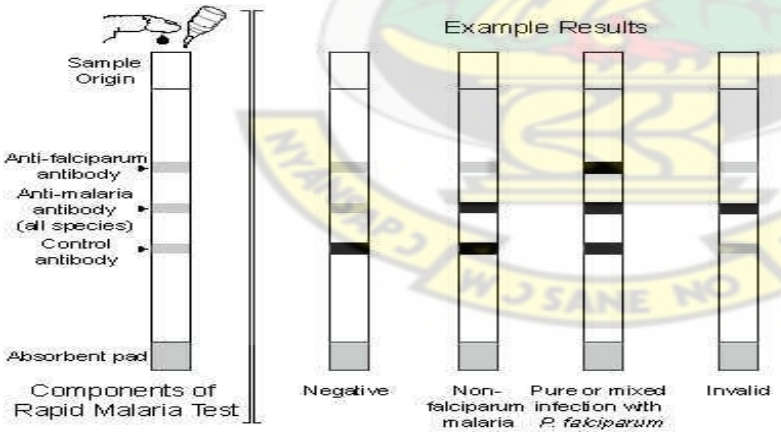

Figure 2.3: Performance of malaria RDT (Kakkilaya, 1990).

2.6.2.1 Antigens targets of malaria RDT

Histidine-rich protein 2 of P. falciparum (PfHRP2): is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the red cell membrane

surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy (Hendriksen *et al*, 2013).

Plasmodium aldolase: is an enzyme of the parasites glycolytic pathway expressed by the blood stages of *P. falciparum* as well as the non-*falciparum* malaria parasites (Joel & Dean, 2013).

Parasite lactate dehydrogenase (pLDH): is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites. It has been found in all 4 human malaria species (Ouattara *et al*, 2011).

Antigens detectable for malaria parasites infection and comparison			
	PfHRP2 tests	PfHRP2 and PMA test	pLDH test
Target antigen	Histidine rich protein 2 of *P. falciparum*,water soluble protein expressed on RBC membrane	Pan-specific *Plasmodium* aldolase. Parasite glycolytic enzyme produced by all species and PfHRP2	Parasite lactate dehydrogenase. Parasite glycolytic enzyme produced by all species
Capability	Detects *P. falciparum* only	Can detect all 4 species	Can detect all 4 species
Cross-reactivity between malarial species	Reported	Reported	Reported
Cross-reactivity with auto antibodies	Reported, high (with rheumatoid factor)	Not known	Reported. low (with rheumatoid factor)

Table 2.1: Antigens detected by malaria RDTs (Joel & Dean, 2013).

2.6.2.2. The sensitivity of malaria RDTs

RDTs for the diagnosis of *P. falciparum* malaria generally achieve a sensitivity of >90% at densities above 100 parasites per µL of blood and the sensitivity decreases markedly below that level of parasite density (Kakkilaya, 1990).

2.6.2.3 Problems with malaria RDTs

False Positivity: False positive results can occur with RDTs due to some reasons. Potential causes for PfHRP2 positivity, persistence of antigens may be due to incomplete treatment or cross reactions with other parasites such as trypanosomiasis (Gillet *et al*, 2013).

False Negativity: The false negative tests have been observed even in severe malaria with parasitaemia>40000 parasites/µl (Bailey *et al*, 2013). Others factors such as the target antigen, the capture antibody, quality variations of test strips, the interpretation of the colour changes can influence the results of malaria RDTs.

2.6.3 Diagnosis of malaria using polymerase chain reaction (PCR)

PCR tests are highly sensitive techniques which can be used for the detection of malaria parasites (Johnston *et al*, 2006). In travellers returning from developing countries, studies based on PCR have been found to be highly sensitive and specific for detecting all 4 species of malaria in cases of low level parasitaemia and mixed infections (Paglia *et al*, 2012). PCR tests are reportedly 10-fold more sensitive than microscopy. PCR tests have also been found useful in the diagnosis of malaria in case of undiagnosed fever (Hopkins *et al*, 2013). These techniques are very costly, limiting their use for malaria diagnosis in malaria endemic areas.

2.6.3.1 Targets of polymerase chain reaction (PCR) tests for malaria diagnosis

These techniques enable the detection of the deoxyribonucleic acid (DNA) and the ribonucleic acid (RNA) (Lilit & Nidhi, 2013) of malaria parasites.

2.6.3.1 Detection of malaria parasites DNA from EDTA blood samples

EDTA is a polyprotic acid containing four carboxylic acid groups and two amine groups with one-pair electrons that chelate calcium and several other metal ions (Madhad & Sentheil, 2014). Calcium removal irreversibly prevents blood clotting within the collection tube. Historically, EDTA has been recommended as the anticoagulant of choice for haematological testing because of its ability to preserve cellular components and morphology of blood cells. The ability of this anticoagulant to stabilize blood for a variety of traditional and innovative tests has amplified its use over recent decades (Banfi *et al*, 2007). EDTA proved not to have an impact on whole blood (Obi *et al*, 2015) for the detection of malaria DNA using PCR (Hodgson *et al*, 2015).

2.7 Comparison of the advantages and disadvantages of malaria RDT, microscopy and PCR in malaria diagnosis.

Characteristics	RDT	Microscopy	PCR
Detection	Detects circulating malaria parasites antigens	Detects the presence or absence of malaria parasites in patient blood smear (thick film	Detects the genome of malaria parasites
Speciation	Cannot differentiate malaria parasites species	Can differentiate malaria parasites species (thin smear)	Can differentiate malaria parasites species (gene specific)
Timing	No time consuming	Time consuming	More time consuming
Sensitivity	Sensitive if parasites density is high	Sensitive if parasites density is high	Sensitive even with low parasites density
Applicability	Applicable everywhere and every time	Not applicable in rural areas (areas of no light)	Not applicable for routine diagnosis
Cost	Cost effective	Expensive	Very expensive
Training	Can be used by everyone with a little training	Needs a skilled trained person	Needs a skilled strained person
Electricity consumption	Does not consume electricity	Consumes electricity	Consumes electricity
Storage	Can be stored at room temperature	Can be stored at room temperature	Can be stored at room temperature

Table 2.3: Comparison of the advantages and disadvantages of methods used in malaria diagnosis (Bhutta et al, 2010; Bharti *et al*, 2008).

2.8 Treatment for malaria

These drugs listed in **Table 2.4** are used in the treatment of malaria. ACT is currently the recommended first line of treatment **(**Dondorp *et al*, 2009; Boyce *et al*, 2015).

Antimalarial policy	Treatments
1st line of unconfirmed malaria	AS+AQ
1st line of *P. falciparum*	AL; AS+AQ
Failure of *P. falciparum*	QN
Severe malaria	QN
Intermittent prevention for pregnant women	SP

Table 2.4: Treatments for malaria (WHO, 2015; Gutman *et al*, 2015; President's Malaria Initiative, 2013a).

The above malaria treatments are also applied in Ghana (Kobbe *et al*, 2008). Herbal medicine are also used for malaria treatment in Ghana and other parts of the world, for either clinical or complicated malaria management (Neergheen-Bhujun**,** 2013; Charlotte & Mohammed, 2012). Some studies showed herbal medicine to be efficient in malaria treatment especially in *P. falciparum* infection (Addae-Kyereme *et al*, 2001; Alex *et al*, 2005).

2.9 Global overview about blood transfusion

Everywhere in the world, blood transfusion is a necessary therapeutic procedure. It is a transferal procedure of blood or blood products from a person called donor, into another person's bloodstream called recipient (Hunter *et al*, 2002).

Transfusion of blood is done in order to save lives mostly in case of severe anaemia, during surgery or severe bleeding (Allain *et al*, 2004). Though it can save lives it can also transmit infectious diseases. It is then necessary to continuously improve the methods used for screening of blood donors.

2.10 Blood transfusion safety in Africa

Blood transfusion safety in Africa is worrisome (Tayou *et al*, 2007). Safe transfusion requires regular or adequate supplies of materials to test blood for infectious diseases, good laboratory testing procedures, adequately trained staffs, presence of quality systems and necessary transfusions (Al-Drees, 2008). It also requires social mobilization to promote voluntary blood donation by sufficient numbers of people who have no infectious diseases that can be transmitted to the recipients of their blood. In Africa many patients do not have access to blood (Tagny *et al*, 2008) though WHO had set target for safe blood in Africa (Bloch *et al*, 2012). Of the estimated 80 million units of blood donated annually worldwide, only 38% are collected in the developing world where 82% of the world's population live. Africa has inadequate blood supply. Shortages of blood at blood bank services result in more death among pregnant women

and children under the age of five years, the majority of which occur in Sub-Saharan Africa (Tagny *et al*, 2008). Furthermore transfusion transmitted infections (TTIs) are prevalent in local African blood donors, contributing to transfusion transmission-related infections to recipients (Bloch *et al*, 2012).

2.11 Transfusion transmitted infections

Blood for transfusion could be a potential source of infection of a variety of transmissible agents (Shimoyama, 2008; Nahom *et al*, 2011). Transfusion transmitted infections (TTIs) are a great concern for patients' safety since the early 1940s (Nabajyoti, 2010; Singh & Sehgal, 2010). The magnitude of TTIs varies from country to country depending on TTIs' loads in that particular population from where blood units are sourced (Chandra *et al*, 2014).

2.11.1 Transfusion transmitted viruses

Blood for transfusion could be infected by viruses such as HIV 1 & 2, HBV, HCV, (Smita, 2015). In Sub Saharan Africa blood donors are routinely screened for the above mentioned viruses (Mavenyengwa et al, 2014). Unfortunately screening is not performed for some viruses such as ZIKV, a mosquito-born flavivirus known to circulate in both Africa and Asia since 1950s (Andrew *et al*, 2012). In some areas of France such as French Polynesia, ZIKV has been found in 42 (3%) blood donors out of 1,505, although asymptomatic at the time of blood donation, were found positive for ZIKV by PCR (Musso *et al*, 2014).

2.11.2 Transfusion transmitted bacteria

Transfusion transmitted bacterial infections (TTBIs) are important complications of blood transfusion (Eder *et al*, 2007; Brecher & Shauna, 2005). The incidence of TTBIs is highly significant than the incidence of transfusion transmitted viruses infections (Hillyer *et al*, 2003). The most common transfusion transmitted bacteria are: Staphylococcus, Streptococcus, Yersinia (Owusu-ofori, 2012). These organisms can cause severe sepsis to the recipient in a

short term after transfusion which can result to death. TTBIs have numerous sources from donor through blood processing to recipient (CDC, 2005).

2.11.3 Transfusion transmitted parasites

The transmission of parasitic organisms through transfusion is relatively rare **(**Gagandeep & Rakesh, 2010). Of the major transfusion-transmitted diseases, malaria is a major cause of transfusion transmitted parasitic infection especially in Sub Saharan Africa (Singh & Sehgal, 2010). Moreover parasitic diseases such as Chagas, Toxoplasmosis, Leishmaniasis can be transmitted through blood (Allain *et al*, 2009). Although the incidence of blood transfusion-transmitted parasitic infections (TTPIs) is lower in comparison to that of bacterial and viral infections; these organisms can pose a considerable risk, especially in immunocompromised individuals.

2.12 Transfusion transmitted malaria

Transfusion transmitted malaria is emerging as a major problem, especially in developing countries (Tagny *et al*, 2010) and in Sub Saharan Africa. The importance of transfusion-transmitted malaria lies in the fact that it can lead to febrile transfusion reaction which can falsely stimulate a haemolytic transfusion reaction especially among non-immune and immune suppressed recipients. It can also lead to the dissemination and spread of malaria drugs resistances. Malaria must always be considered in any patient with a febrile illness at post-transfusion (Bahadur *et al*, 2010). In United States, donors who have had malaria are not permitted to donate for three years after they become free of symptoms (Al-Drees, 2008). Because of these control measures, transfusion transmitted malaria (TTM) in the United States is uncommon (Mungai *et al*, 2001; Goodnough, 2003). Ninety-five per cent (95%) of transfusion in Sub Saharan Africa involve whole blood rather than components. Whole blood and red blood cells concentrates are the commonest sources of TTM (Jobert *et al* 2013, Owusu-

Ofori *et al*, 2013). Hence the control of malaria in endemic areas and transfusion safety are important aspects to be examined (Kátia *et al*, 2014) by considering the diagnosis of malaria routinely in recipients at post transfusion (Kitchen & Chiodini, 2006; Bruneel *et al*, 2004).

2.12.1 Prevalence of malaria in donors

In Sub Saharan Africa, blood donors are not screened for malaria. The prevalence of malaria in blood donors differs from country to country. The prevalence of malaria in blood donors is higher in some countries such as Cameroun (65%) (Noubouossie *et al*, 2012) and lower in some such as Ghana (13%) (Muntaka & Opoku, 2013). These studies were conducted at different areas and different periods of time.

Determining the prevalence of malaria in blood donors may not show the actual danger of TTM because in malaria endemic areas most people may have immunity against the disease malaria.

2.12.2 Incidence of transfusion transmitted malaria

The real incidence of transfusion-related malaria is unknown in some part of the world (Kátia *et al*, 2014). However a study conducted in Ghana suggested the incidence of transfusion transmitted malaria (TTM) in recipients to be of 14% to 28% through genotyping (Freimanis *et al*, 2013).

2.12.3 Donors screening for malaria

A lot of consideration have been put into transfusion transmitted viruses infections (Vamvakas & Blajchman, 2009; Regan & Taylor, 2002). Less is done about parasites transmission and emerging viruses such as West Nile virus (Busch *et al*, 2003) especially in Sub Saharan Africa where malaria transmission through blood remains an issue (Tagny *et al*, 2010). Blood donors, in Sub Saharan Africa are not tested for malaria despite recommendations that, all blood should be tested for malaria (Mark & Shauna, 2005; Zhao *et al*, 2012). Numerous reasons exist, why

it is difficult to routinely screen blood donors for malaria in Sub Saharan Africa (Tayou *et al*, 2007). Some of the reasons include severe blood shortages which are widespread and will be exacerbated by rejecting blood that contains malaria parasites. Furthermore, the lack of appropriate screening assays for malaria in blood banks limits the diagnosis of the disease and hamper the blood safety (Yang & Rothman, 2004). The possible destruction of the malaria parasites in blood bags and the anti-malarial treatment given to recipients at post transfusion are the described possibilities to prevent TTM. However a careful screening of donors according to the recommended exclusion guidelines remains the best way to prevent TTM (Mungai *et al*, 2001).

2.12.4 Post transfusion malaria

Post transfusion malaria may be defined as malaria occurring after transfusion. It includes, getting malaria through blood transfusion, called TTM, or through environmental infection which surfaces after transfusion.

When malaria is transmitted through blood to a non-immune recipient, it can be rapidly fatal (Kátia *et al*, 2014). Most young infants in malaria endemic areas who have not had repeated exposure to the parasite may be regarded as non-immune recipients. Therefore they may be susceptible to TTM as a non-immune person who lives in a non- malaria endemic area (Idro *et al*, 2005). Post transfusion malaria may lead to severe complications of malaria especially in case of *P. falciparum* infection. There is necessity to supply adequate blood in order to reduce malaria mortality and morbidity in Sub Saharan Africa especially among children under 5 years old.

CHAPTER 3: STUDY METHODOLOGY

3.1 Study site

The study was conducted at the Komfo Anokye Teaching Hospital (KATH) Kumasi. Kumasi is the capital city of the Ashanti region of Ghana.

KATH is the second largest hospital of Ghana. It is situated at Bantama a suburb of Kumasi in Ashanti region. The hospital offers services in 11 departments which are: Anaesthesia and Intensive Care Unit (ICU); Child Health; Dental, Eye, Ear, Nose and Throat (DEENT); Diagnostics; Medicine; Obstetrics & Gynaecology; Oncology; Polyclinic; Surgery; Accident & Emergency department and Pharmacy. The transfusion services in the hospital are provided by the Transfusion Medicine Unit (TMU).

3.1.2 Transfusion Medicine Unit

The Transfusion Medicine Unit (TMU) in KATH, obtains blood, stores and distributes it in the hospital. It is composed of three units which are, donor organization, donor care and the laboratory. TMU is currently only able, to provide whole blood, concentrated red cells (packed cells) and fresh frozen plasma for transfusion. It does not provide components such as platelets and cryoprecipitate.

About 15, 000 transfusions are carried out in KATH each year. Blood is collected from 2 distinct populations of donors, voluntary (70%) non-remunerated blood donors and replacement donors. Blood donors are screened for HIV, HBV, HCV, and Syphilis but not for malaria (Transfusion, 2012). After the collection of blood from blood donors, the blood is sent to the laboratory unit where grouping is performed follow by storage. In case of any request for blood transfusion, grouping is performed on the recipient's blood sample and the corresponding donor's blood, follow by cross matching and delivery. The percentage of blood distribution in KATH among the different departments, is high in Obstetrics and Gynaecology (25%), follow

by Accident and Emergency (24%). Paediatric department comes third with a percentage of 19% (Owusu-Ofori, 2012).

3.1.3 Child Health Department

It is constituted of six wards, which are: Paediatric Emergency Unit (PEU), B5, C5, B4, Mother and Babies Unit (MBU) and Paediatric Intensive Care Unit (PICU). It receives children from 0 to 12 years old with PEU being the first point of call in case of any emergency. In general, PEU admits all medical cases, B5 admits haematology and oncology cases, C5 admits respiratory and cardiology cases, B4 admits malnutrition and renal cases, MBU admits neonates and babies cases right from delivery till they are less than two months and PICU admits critically ill children.

3.2 Recruitment and enrolment process

3.2.1 Inclusion criteria

Children under five years old from the Child Health Department expect PICU who were receiving blood transfusion at the Child Health Department of KATH between the periods of November 2014 to February 2015 were recruited into the study.

3.2.2 Exclusion criteria

All children under the age of five years old receiving blood in other Departments other than Child Health Department (PEU, B4, B5, C5, and MBU) were not included.

All target population who were transfused with plasma.

All target population who refused consent.

3.2.3 Enrolment process

- Step 1: Any child fulfilling the inclusion criteria and who was expected to receive blood was followed up for enrolment.

- Step 2: In the Ward, children were identified and study explained to the relative in his/her understanding language.

- Step 3: When the relative was satisfied with the explanation consent was then obtained through signature or finger print on a consent form provided.

- Step 4: Child is not enrolled if relative did not consent.

3.3 Study design

The study was a cohort study.

3.4 Data collection

Data used in this study were obtained as primary data from transfused recipients. Data was entered and analysed in Excel Microsoft 2013. For this study, any recipient who gave consent was enrolled and data was collected from the day of enrolment. Information gathered included name of the recipient, age, sex and reason for admission. Any malaria diagnosis case was reported from the folder of the recipient as well as the methodology used for the diagnosis (RDT or microscopy). Anti-malarial drugs administrated before and after blood transfusion were also reported. Symptoms were recorded from recipients' folders at post transfusion. Data was also recorded in case of any situation preventing the collect of the second sample at post transfusion.

3.5 Sample size calculation

A previous study done in Ghana (Owusu ofori, 2012) had a prevalence of TTM of 13.7% by RDT. Using a confidence interval of 95% the sample size for this study was calculated as 182 using the formula $n = Z^2 P \frac{(1-P)}{d^2}$.

However a number of 179 samples were collected during the study.

n = sample size

Z = Z statistic for a level of confidence = 1.96 (for 95% CI)

P = prevalence or proportion = 0.02 (2%)

d = precision = 0.05 (5%)

$n=Z^2P\ (1-P)/d^2$

n= 1.96 x 1.96 x 0.137 x (1-0.137)/0.05x0.05

n= 182

3.7 Quality assurance and quality control

To ensure the quality of the slide, new microscopy slides were used and a newly Giemsa Stain prepared.

Malaria RDT was stored at room temperature in order to preserve its sensitivity because high temperature could have destroyed it sensitivity.

Negative and positive controls were used for all PCR reactions in order to ensure the quality of the reagents and free contaminations.

During the laboratories works, standard procedures were followed for each test method to ensure coherence.

3.7 Blood samples collection

Blood sample collection from recipients registered into the study

The recipients were subjected to blood transfusion after a laboratory diagnosis confirming the need for blood transfusion. Recipient's blood sample was then sent to the laboratory unit of the blood bank service where grouping, cross matching with donors blood and delivery of the compatible donor's blood were done. After enrolment of a child expected to receive blood for transfusion, 1ml of blood was drawn before the child received the transfusion. Blood collection was done with a 2ml sterile syringe or a sterile lancet, either through venous, heel or third finger prick. The blood was transferred into an EDTA tube. The tube was then labelled (e.g.: P1=recipient number 1 enrolled in the study) and sent to the laboratory for malaria testing using

malaria RDT, microscopy and PCR. Within 48 hours after blood transfusion the same procedure was performed on the same recipient. Post transfused blood was collected, transferred into an EDTA tube, labelled (e.g.: 2P1= second sampling of recipient number 1) and sent to the laboratory for malaria diagnosis.

Blood sample collection from blood donors

About 1ml of donor's blood, which was going to be transfused to the corresponding recipient, was collected from the laboratory section of the TMU. The blood was transferred into an EDTA tube labelled with the same number of the corresponding recipient (e.g.: B1= blood given to P1) and sent to the laboratory for malaria testing.

3.8 Blood samples testing methods

3.8.1 Blood samples processing for malaria diagnosis using malaria RDT.

In the laboratory, about 5µl of blood from the labelled EDTA tube, was taken with a pipette provided with the kit. The pipetted blood was loaded into the sample well of the RDT. Two drops of provided buffer was loaded into the buffer well of the same RDT. The result was then read within 20 minutes. The same procedure of the RDT was performed on sample collected from blood donors and recipients (at post transfusion). When in addition to the control line, a pink/ red line appeared at the *P. falciparum* region and the test was reported as *P. falciparum* positive. When the line appeared at the pan region the test was reported as pan positive, and it was reported as *P. falciparum* & pan positive when 2 distinct lines appeared at the *P. falciparum* and pan's regions. A negative result was recorded when there was no additional line to the control line. No invalid result was recorded. An invalid result would have been reported if there was no line seen. These procedures were performed following the manufacturer instructions. (**Figure 3.1**).

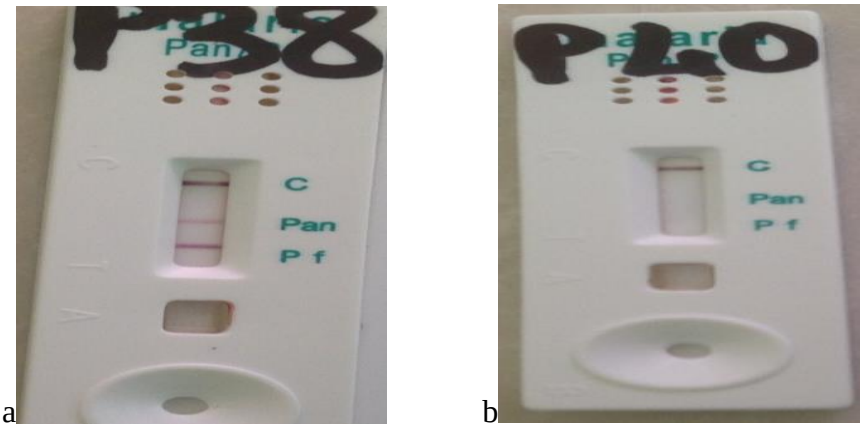

Figure 3.1: Sample of malaria RDT result; a = *P. falciparum* and pan positive, two distinct lines appeared at the *P. falciparum* (*P. f*) region and pan region in addition to the control line; b = negative result, only the control line showed no line at the *P. falciparum* region neither the pan region hence malaria negative result.

3.8.2 Blood samples processing for malaria diagnosis using microscopy

Thick and thin blood smears were prepared in the laboratory from the same blood used for the malaria RDT. A pipette was used to transfer two distinct drops of blood sample from a labelled EDTA tube unto a clean glass slide. Thick and thin films were prepared on the same slide. The slide was labelled appropriately with the code attributed to the EDTA tube. Thick film was used to detect the presence or absence of malaria parasites and thin film was used to identify the malaria species.

Thin film:

A drop of blood was placed in the middle of a clean glass slide. Another glass slide with clean and smooth end was placed in contact with the drop of blood forming an angle of 45° to enable the blood to spread along the edge of the second glass slide which acts as the spreader. The spreader was pushed forward to the end while maintaining the angle. This ensured that the blood was spread thinly. The slide was air dried and fixed by dipping into 100% methanol for about 10 seconds and then air dried again. The smear was then stained with a freshly prepared 5%

Giemsa stain solution for 45 minutes and washed with clean distilled water. The slide was then placed on a drying rack and allowed to air dry.

Thick film:

A drop of blood was placed at the bottom of the prepared thin smear. Using the corner of another clean and smooth glass slide, the drop of blood was spread in a circular way to ensure the spread of the blood with moderate thickness. The blood was air dried without being fixed in methanol. The smears were then stained with a freshly prepared 5% Giemsa stain solution for 45 minutes and washed with clean distilled water. The slide was then placed on a drying rack and allowed to air dry. The smears (thin and thick) were examined with 100X objective.

Figure 3.2: Microscopy slides (thick and thin smears).

3.8.3 Blood samples processing for Plasmodium falciparum DNA detection using PCR.

After performing the malaria RDT and microscopy on the pre transfusion, donors and post transfusion samples, the rest of the blood samples were stored in a freezer at -20°C. A number of 96 samples were purposely selected for PCR. They were composed of 32 recipients' blood samples at pre transfusion, their corresponding 32 donors' blood and their 32 blood samples at post transfusion.

3.8.3.1 Extraction of DNA from blood sample

The extraction of DNA was performed on the selected 96 samples using the Genotype DNA isolation kit (n° 51548, Hain Life science, Germany). The DNA was extracted from 50 µl of

whole blood by following the manufacturer's instructions. The purified DNA was then stored at -20°C.

3.8.3.2 Plasmodium falciparum DNA identification

The 96 extracted DNA samples were tested by PCR, targeting the *Plasmodium* small subunit ribosomal RNA (ssrRNA) gene, with 664-693 indicating the position of the forward primer (FAL 1) and 840-869 indicating the position of the reverse primer (FAL 2) for the detection of *P. falciparum* DNA. The PCR was composed of two amplification cycles.

The first amplification was called primary PCR. It was done in order to increase the quantity of template for the nested PCR which was the second amplification cycle. The primary PCR amplified the DNA targeting all the human plasmodium species including *P. falciparum*.

The second PCR called nested PCR was specific only to *P. falciparum*. The product of the primary PCR was then used to amplify only the gene specific to *P. falciparum.*

For the first amplification cycle called primary PCR, 42.5µl of the master mix 1 was prepared for the testing of 1 sample. The reagents were brought out from the freezer (-10°C) thawed at room temperature and shortly centrifuged before they were used. The master mix 1 was composed of following reagents in **Table 3.1** for testing of 1 sample.

Reagents	Volume (µl)
Buffer	10
Magnesium	8
dNTPs	4
rPLU1 (forward) 5'TCAAAGATTAAGCCATGCAAGTGA3'	5
rPLU5 (reverse) 5'CCTGTTGTTGCCTTAAACTCC3'	5
Tap polymerase	0.25
Nuclease free water	10.25
Total	42.5 µl

Table 3.1: Composition of the master mix 1 for the primary PCR.
rPLU1 and rPLU5 indicate the primers used in the primary PCR for the amplification of the DNA to all the plasmodium species. dNTPs indicates dinucleotide triphosphates.

The solution of 42.5 µl of the master mix 1 was shortly centrifuged and transferred into a labelled PCR's tube. Afterward, 7.5µl of the extracted DNA (sample) was added to the 42.5µl

of the master mix 1. The DNA amplification was then performed into a thermal cycler with the following steps; initial denaturation (94°C, 4min), a cycling of 5: denaturation (94°C, 30s), hybridization (60°C, 1min), elongation (72°C, 1min), a cycling of 30: denaturation (94°C, 30s), hybridization (55°C, 1min), elongation (72°C, 1min), followed by a final elongation (72°C, 4 min) and a conservation stage (10°C, unlimited time). For the nested PCR, a master mix 2 was prepared. It was composed of the following reagents in **Table 3.2** for the testing of 1 sample.

Reagents	Volume µl
Buffer Blue	4
Magnesium	3.2
dNTPs	1.6
FAL 1 (forward) 5'TTAAACTGGTTTGGGAAAACCAAATATATT3'	2
FAL 2 (reverse) 5'ACACAATGAACTCAATCATGACTACCCGTC3'	2
Taq polymerase	0.1
Nuclease free water	5.1
Total	18 µl

Table 3.2: Composition of the master mix 2 for the nested PCR. FAL 1 and FAL 2 indicate the primers used for the amplification of only *P. falciparum* gene. dNTPs indicates dinucleotide triphosphates.

The solution of 18µl of the master mix 2 was shortly centrifuged and then transferred into a labelled PCR's tube. Afterward the 2 µl of the product of the primary PCR which was the master mix 1 was added to the labelled PCR's tube containing the master mix 2. The DNA amplification was then performed into a thermal cycler with the following steps; initial denaturation (94°C, 4min), a cycling of 5: denaturation (94°C, 30s), hybridization (63°C, 1min), elongation (72°C, 1min), a cycling of 30: denaturation (94°C, 30s), hybridization (58°C, 1min), elongation (72°C, 1min) followed by a final elongation (72°C, 4min) and a conservative stage (10°C, unlimited time). The final product of the PCR was then sent to the Gel room for electrophoresis and bands' detection.

3.8.3.3 Electrophoresis and band detection

The final product of the PCR was detected through electrophoresis on 1.5% agarose gel. The casting tray was prepared and the comb fixed in position. An amount of 1.2 g of agarose gel were weighted and poured in a volume of 80ml of 1xTris-borate with EDTA (TBE composed of Tris base, Boric acid and EDTA). The solution was mixed well and heated for 2 minutes,

after its temperature was cooled down under running water. When the gel had cooled to the point that it could be comfortably touched, 3 µl of ethidium bromide was added and uniformly mixed with the agarose. The gel was carefully poured into the casting tray. All air bubbles were removed and the gel allowed to solidify. After solidification the comb was removed and the gel was placed in the electrophoresis tank. A volume of 8 µl of the final product of the PCR was loaded into the wells. Afterward, 5µl of 100 bp ladder (Invitrogen) was loaded into the first well (starting from the left). The positive and negative controls were the last to be loaded. Then an electric current of 100 V were applied to the gel for 30minutes. Image of the gel was captured, using a Trans-illuminator, with the software infinity Vx2. The bands were detected under UV light (**Figure 3.3**).

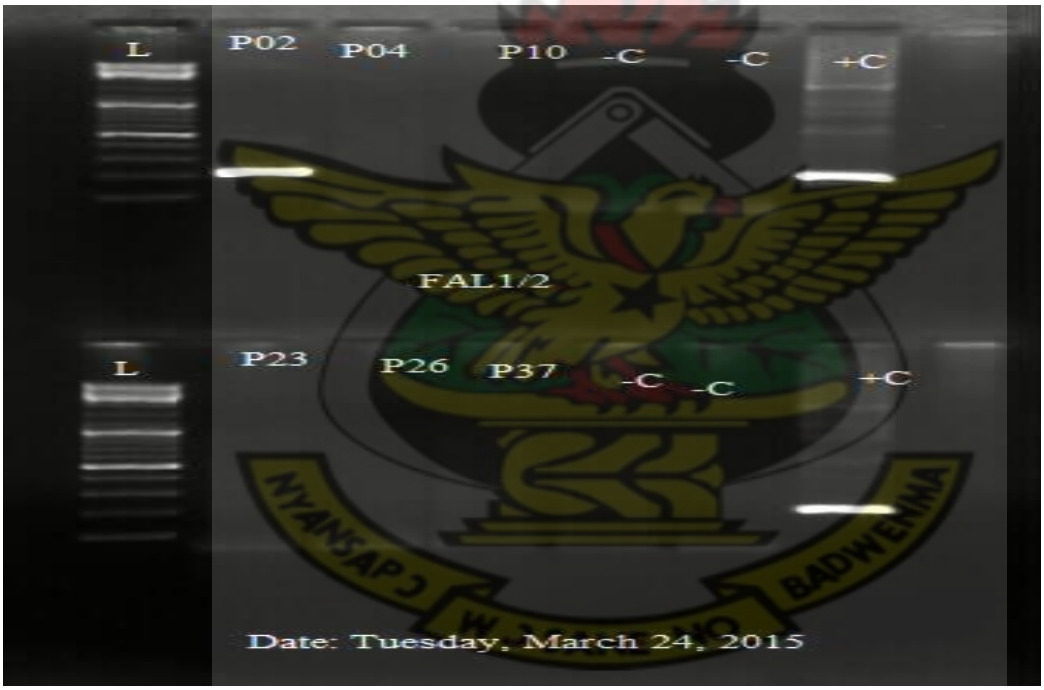

Figure 3.3: 1.5% agarose gel analysis of nested PCR product.
(L= ladder; P02, P04, P23, P26 and P37= pre transfusion samples of patients number 02, 04, 23, 26 and 37 respectively; -C= negative control; +C=positive control; FAL= falciparum)

3.9 Blood samples selection for determination of the prevalence, incidence and efficiency

3.9.1 Determination of the prevalence of malaria

Prevalence of malaria was determined independently using malaria RDT and microscopy on 179 pre-transfusion samples, 179 donors' samples and 121 post transfusion samples.

3.9.2 Determination of the incidence of post transfusion malaria

Incidence of malaria at post transfusion was determined on 32 recipients' blood samples using PCR, due to its high sensitivity and specificity compared to microscopy and malaria RDT.

All the 32 recipients tested negative at pre-transfusion, 30 of them turned positive at post transfusion and the other 2 remained negative using malaria RDT.

3.9.3 Determination of the efficiency of malaria RDT

The results of the PCR were used to determine the efficiency of malaria RDT to microscopy at post transfusion using the online statistic calculator, Vassar Stats with 95% confidence interval. The sensitivity (SENS), specificity (SPES), positive predictive value (PPV) and negative predictive value (NPV) were calculated using the following formula:

Sensitivity = TP / (TP+FN)

Specificity = TN / (TN+FP)

Positive predictive value = TP / (TP+FP)

Negative predictive value = TN / (TN+FN)

3.10 Ethical consideration

Ethical approval was obtained from Ethics Committee on Human Research and Publication of the School of Medical Sciences (SMS) Kwame Nkrumah University of Science (KNUST). Certificate and approval to undertake the research at the study site was also obtained.

Recipients enrolled into the study gave their consent after a clear verbal explanation of the study. Parents who were literate were given the consent forms to read by themselves. Enrolled recipients had their samples collected with precaution in order to make sure that the recipients were not going to get any infection back, or feel a severe pain.

CHAPTER 4: RESULTS

Recipients' characteristics

A total of 179 children under five years old were recruited into the study. The majority (44%) were from MBU (**Table 4.1**). The male children were in the majority (64%).

The ages ranged between 0 - < 1 year (52%), 1 - < 2 years (12%), 2 - < 3 years (18%), 3 - < 4 years (5%) and 4 - < 5 (12%). The mean age was 1.37 years old.

At the time of enrolment, 76 recipients (42%) had been tested for malaria in the wards using malaria RDT (1st response malaria RDT) which detected only *P. falciparum* infection (**Table 4.1**). Among those that tested negative, 17% were given anti-malarials, contrary to WHO treatment guidelines. There were 103 (58%) who were not tested for malaria in the wards. Contrary to WHO guidelines, 17% were also given anti-malarials. The most frequently used anti-malarial drugs was Artemisinin-based Combination Therapy (ACT) (74%) followed by camoquine (18%), artemether Lumefantrine (6%) and quinine (2%).

Characteristics		N (%)
Wards	MBU	79 (44%)
	PEU	62 (35%)
	Others	38 (21%)
Sex	Male	115 (64%)
	Female	64 (36%)
Recipients tested for malaria		76 (42%)
	Malaria positives	41 (54%)
	Treatment with anti-malarial	41 (54%)
	Malaria negative	35 (46%)
	Treatment with anti-malarial	6 (17%)
Recipients not tested for malaria		103 (58%)
	Treatment with anti-malarial	17 (17%)

Table 4.1: Characteristics of the 179 recipients recruited into the study.

Study drop out

Post transfusion samples could not be collected from 58 (32%) of those enrolled. Death (36%) accounted for the major reason why these samples were not collected (**Table 4.2**). Seven (12%) who were due to be transfused were no more transfused as required. There were 6 recipients (10%) who were transfused twice the same day. Another group of 6 children (10%) could not be traced after transfusion. Five children (9%) were discharged the same day after blood transfusion. A group of 4 children (7%) had their clinical condition deteriorating, therefore taking further blood sample was deemed unethical. Only 1 (2%) was wrongly enrolled into the study, the correct age of 6 years was discovered after the enrolment (**Table 4.2**).

Reasons of drop out	N (%)
Death	21 (36%)
Blood transfusion stopped	7 (12%)
Consent withdrawals	6 (10%)
Transfused twice the same day	6 (10%)
Could not traced after blood transfusion	6 (10%)
Discharged before second sample collection	5 (9%)
Clinical condition worsened	4 (7%)
Wrongly enrolled	1 (2%)

Table 4.2: Reasons for why recipients did not have a post transfusion sample collected.

Prevalence of malaria

Among the 179 pre-transfusion samples collected from recipients, prevalence of malaria by malaria RDT was 34% (60/179). Out of those 60 positive samples, 19 (32%) were mixed infection for *P. falciparum* and *P. ovale* or *P. malarae* or *P. vivax*. The other 41 (68%) samples were only *P. falciparum* positive. The prevalence of malaria by microscopy was 13% (24/179) (**Table 4.3)**. *P. falciparum* was the only species identified after speciation when light microscopy was used.

The prevalence of malaria among the 179 samples collected from blood donors, using malaria RDT was 27% (49/179) (**Table 4.3)**. Among those positive, 46 (94%) were *P. falciparum* positive, 2 (4%) were mixed infection with *P. falciparum* and *P. ovale* or *P. malarae* or *P.*

vivax, 1 (2%) was mixed infection with *P. ovale* or *P. malarae* or *P. vivax*. The prevalence of malaria by microscopy was 7% (12/179) and *P. falciparum* was the only species identified.

There were 121 post-transfused samples tested for malaria. The prevalence of malaria at post transfusion, by malaria RDT was 59% (71/121). Out of the 71 positives, 2 (3%) were mixed infection with *P. falciparum* and *P. ovale* or *P. malarae* or *P. vivax*. The other 69 (97%) were only *P. falciparum* positive. The prevalence of malaria by microscopy was 4% (5/121) (**Table 4.3**).

	Test methods	Number of samples tested	Number of positive	Prevalence
Pre transfusion	RDT Microscopy	179 179	60 24	34% 13%
Donors	RDT Microscopy	179 179	49 12	27% 7%
Post transfusion (within 48H after transfusion)	RDT Microscopy	121 121	71 5	59% 4%

Table 4.3: Comparison of the prevalence of malaria by malaria RDT and microscopy.

Incidence of post-transfusion malaria.

The incidence of post transfusion malaria was determined from the 32 selected recipients diagnosed for *P. falciparum* infection using PCR. Among the 32 recipients, 28 were negative at pre transfusion, 21 of their corresponding donors' blood were *P. falciparum* positive and 16 became positive at post transfusion. At post transfusion, the condition of those 16 recipients was recorded from their folders. Only 1 (6%) was reported to be in critical condition, 10 (63%) were febrile and pale and no symptoms was recorded on the other 5 (31%) recipients. The incidence of post transfusion malaria by PCR was 57% (16/28). Among the other 5 recipients, though they received positive *P. falciparum* blood, were negative at post transfusion. Three were treated with ACT at post transfusion. The other 2 were also negative at post transfusion though they did not receive any anti-malarial treatment.

They were 7 recipients out of the 28 were negative at pre transfusion, received negative *P. falciparum* blood and remained negative at post transfusion.

Determination of the efficiency of malaria RDT to microscopy in detecting malaria at post transfusion.

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With PCR as the gold standard, the sensitivity of malaria RDT in detecting *P. falciparum* at post transfusion was 100% compared to 5.2% by microscopy. However microscopy had the highest specificity of 100% while malaria RDT had 15.3%. The negative predictive value was 100% by RDT while microscopy had a 100% of positive predictive value (**Table 4.4**).

Post transfusion (within 48H after transfusion)	**PCR**		**Sensitivity % (95%CI)**	**Specificity % (95%CI)**	**Positive predictive value % (95%CI)**	**Negative predictive value % (95%)**
	+ve	**-ve**				
RDT						
+ve	19	11	100	15.3	63.3	100
-ve	0	2	(79 - 100)	(2.7 - 46.3)	(43.9 - 79.4)	(19.7 - 100)
Microscopy						
+ve	1	0	5.2	100	100	41.9
-ve	18	13	(0.2 – 28.1)	(71.6 - 100)	(5.4 - 100)	(25.1 - 60.1)

Table 4.4: Comparison of the efficiency of malaria RDT to microscopy in detecting post transfusion malaria.

CHAPTER 5: DISCUSSION

5.1 Incidence of post transfusion malaria

The incidence of post transfusion malaria by PCR was 57% in single transfused recipients. This was high comparing to the incidences of 2.9% of post transfusion malaria observed in single transfused recipients and 4.9% in multiple transfused recipients in Sudan, shown by Ali et al, (2004) where microscopy was the only test method. The differences could be due to the sensitivity of the test methods used because, PCR is more sensitive than microscopy.

The results of this study shows that, post transfusion malaria is a real fact and must be taken into consideration. It could have an impact on the health of non-immune children leading to complications such as severe anaemia or death (Jobert *et al*, 2013).

Among those 21 recipients who received *P. falciparum* infected blood, 5 recipients were negative at post transfusion. Three of them received ACT as anti-malarial at post transfusion. This anti-malarial drug is known to be the fastest acting anti-malarial available. It inhibits the development of the trophozoites and thus prevents progression of the disease. Young circulating parasites are killed before they sequester in the deep microvasculature. This drug starts acting within 12 hours. These properties of the drug are very useful in managing *P. falciparum* malaria (Kakkilaya, 1990). This could explained why the 3 recipients were detected negative within 48 hours at post transfusion though they received positive donors' blood.

However, anti-malarial drugs did not influenced the results of malaria RDT at post transfusion. This means that, recipients who were negative at pre transfusion were detected malaria positive at post transfusion by malaria RDT after receiving a positive malaria donors' blood.

The post transfusion malaria cases in this study were likely to be of TTM because all the 16 positive *P. falciparum* post transfused recipients were initially negative at pre transfusion and became positive after being transfused with a positive *P. falciparum* blood. This show that

children under five years old in KATH could be at risk of TTM which can led to severe complications of malaria because of their low level of immunity against the disease, malaria.

Among the 32 samples subjected to PCR, 7 were negative at pre transfusion, received negative *P. falciparum* blood and remained negative at post transfusion. Based on these results showed, no likely environmental post transfusion malaria case was detected in this study.

5.2 Prevalence of malaria in blood donors

The prevalence of malaria by malaria RDT was 27% in blood donors, while it was 7% by microscopy. The presence of *Plasmodium* in transfused blood represents a potential risk of infection transmission by blood.

This study did not measure actual disease transmission and neither was the viability of the parasites determined so it is not possible to measure the actual risk of transmission. But as seen by the incidence rate (57%) of post transfusion malaria at least half of the recipients will become positive for malaria after transfusion.

In this study, the prevalence of malaria in blood donors by RDT was high (27%), contrary to what was found by Laban *et al* (2015) (1.3%) in Zambia. The PfHRP-2 RDT kit was their RDT of choice. The difference between the prevalence could be due to the different endemicity of malaria in the areas the studies took place. The prevalence was similar to what was obtained by Owusu-Ofori (2012) (13.7%) in Ghana. In that study the First Response malaria RDT which detected the HRP2 of *P. falciparum* was the RDT of choice.

The prevalence of malaria in blood donors (7%) by microscopy in this study was similar to that of Adeoye *et al* (2014) (15.4%) in Lagos State, Nigeria during the dry season. Thin and thick films were performed and stained with Giemsa stain. Damen *et al*. (2015) had a prevalence up to 65.1% during the raining season in North-Central Nigeria, where microscopy was the diagnosis tool. In Ghana blood donors are not screened for malaria despite WHO recommendations (Owusu-Ofori *et al*, 2010).

5.3 Prevalence of malaria in patients prior to transfusion

In the wards, malaria accounted for 22% of the admitted patients enrolled into the study. The prevalence of malaria in patients prior to transfusion by malaria RDT was 34% while microscopy was 13% among children under five years old. This showed that malaria is still one of the major causes of children admission in KATH.

The prevalence of malaria in children under five years old by microscopy (13%) in this study was lower compared to that of Gay *et al* (2014) (52%) among admitted children under the age of five in Niger. Smears microscopy stained with Giemsa stain was their test method. The study was performed during the raining season (July-October). These differences could be due to the different seasons the studies took place. However the prevalence was similar to that of Bryce *et al* (2005) (8%) among admitted children under the age of five in some few countries in Asia and Africa.

The prevalence of malaria by RDT (34%) was similar to that of Konaté *et al* (2011) (26.4%) in children under the age of five in Burkina Faso. The study was done during the raining season. The malaria RDT used was able to detect the HRP2 of *P. falciparum.* The prevalence of 13.3% of malaria in OPD children under five years in Zambia, showed by Manyando *et al* (2014) was lower comparing to the prevalence obtained by RDT in this study. The malaria RDT used was made to detect the HRP2 of *P. falciparum*.

5.4 Reasons of the difference between the prevalence of malaria in blood donors and patients prior to transfusion.

The prevalence of malaria in patients prior to transfusion by malaria RDT was 34% and 13% by microscopy. In blood donors, the prevalence by malaria RDT was 27% and 7% by microscopy. In both test methods the prevalence is higher in patients prior to transfusion than blood donors. Blood donors were not physically sick and did not show any malaria symptoms hence they could have a low parasitaemia, while the admitted patients were already sick.

5.5 Sensitivity of malaria RDT and microscopy

In post transfused samples, malaria RDT had a sensitivity of 100% while microscopy had 5.2%. In this study, the sensitivity of 100% showed by malaria RDT conformed to WHO recommendations for malaria RDT performance (>90%) (Chou *et al*, 2012). Endeshaw *et al*, (2008) found that HRP-2 based *P. falciparum*-specific tests, generally have good sensitivity (over 90%). A similar study on the efficiency of RDT, showed malaria RDT to be more sensitive (100%) than microscopy (91%) (Gatti *et al*, 2007). A sensitivity of 98% of malaria RDT was shown in a study done by Bahadur *et al* (2010) in India.

Over all malaria RDTs are sensitive for malaria diagnosis comparing to microscopy (Akotet *et al*, 2014; Chanda *et al*, 2011) either in malaria diagnosis or malaria cases management (Asante *et al*, 2010) in malaria endemic areas such as Ghana (Daniel *et al*, 2013). Malaria RDTs offer higher sensitivity (>90% for *P. falciparum*) than microscopy (Weekley & Smith, 2013).

Although microscopy remains the gold standard for malaria diagnostic (Li *et al*, 2013; Masanja *et al*, 2012), RDTs offer a good alternative (Mens *et al*, 2007).

5.6 Feasibility of the implementation of malaria RDT

In this study, malaria RDT had shown high sensitivity. If implemented as a screening tool it will efficiently be able to detect parasitaemia. What becomes of malaria positive blood donors or? We cannot afford to waste blood, because of the already severe blood shortages in malaria endemic areas (Bates et al, 2011) and the transfusion of a known infected blood could result in ethical issues. Consideration also as to be made whether the positive malaria blood donors will be treated or not or if they will have to buy their own medicine. Malaria RDT detects the antigen of *P. falciparum* even in the absence of viable parasites. These are some of the challenges in implementing malaria RDT for use at blood bank services.

According to Masanja *et al*, (2012) and Boyce *et al* (2015), the implementation of malaria RDT at post transfusion will increase the use of RDT for malaria confirmation and reduce the

prophylaxis treatment with ACT given to recipients at post transfusion without any laboratory diagnosis, which goes against WHO suggestion (Newman, 2012). Besides Gerstl *et al*, (2010) supported the wider use of malaria RDT to accurately diagnose malaria and avoid over and under treatment of malaria. They also refuted the idea that, malaria RDT may miss a significant number of true positive among children under five years old. An effective treatment of malaria, depends on a laboratory diagnosis (Akotet *et al*, 2014).

The implementation of malaria RDT testing at post transfusion will improve differential diagnosis & fever management, ensure prompt treatment of recipients, diminish unnecessary use of ACTs and restrict the spread of malaria drugs resistances, (Zhao *et al*, 2012**)**. Also it will reduce the administration of malaria drugs without diagnosis in Ghana (Ansah *et al*, 2015), reducing recipients' expenditures (Asante *et al*, 2010) and the risk of children exposed to unnecessary treatment (Reyburn *et al*, 2007).

5.7 Presumptive anti-malarial treatment at post transfusion

The practice in KATH shows that some 17% of malaria negative transfused recipients and similarly 17% of those not investigated for malaria were given anti-malarial treatment.

This practice in KATH was also confirmed by Owusu-Ofori (2012) where anti-malarial treatment were given despite a negative laboratory test. This goes against WHO recommendations which suggest treatment after laboratory confirmation (Boyce *et al*, 2015). These results showed that the recommendations are not always met by the physicians. However studies are needed to understand why this practice continue.

5.8 Comparison of humans Plasmodium antigens

The HRP2 is water-soluble protein expressed only by *P. falciparum* trophozoites. It persists in the circulating blood after the parasitaemia has cleared or has been greatly reduced. It generally takes around two weeks or months after successful treatment for HRP2-based tests to turn

negative (Hendriksen *et al*, 2013). Since HRP-2 is expressed only by *P. falciparum*, these tests will give negative results with samples containing only *P. vivax*, *P. ovale*, or *P. malariae*. Studies need to be done on how a positive HRP2 result must be interpreted.

Plasmodium lactate dehydrogenase (pLDH) is an enzyme. PLDH does not persist in the blood but clears about the same time as the parasites following successful treatment (Ouattara et al, 2011). The lack of antigen persistence after treatment makes the pLDH test useful in predicting treatment failure. It can be used to detect infection caused by *P. vivax*, *P.malariae*, and *P.ovale* and *P.falciparum*. PLDH detection seems to be the best in the diagnosis of malaria.

Plasmodium aldolase is an enzyme produced by all four species (Joel & Dean, 2013). The presence of antibodies against the plasmodium aldolase in the sera of human adults partially immune to malaria suggest that plasmodium aldolase is implicated in protective immune response against the parasite.

5.9 Study limitation

The limitation to this study could have been:

1. The sample selection for PCR was purposely done to enable incidence to be measured. This may be a selection bias and may had affected the interpretation of the results of microscopy comparing to that of malaria RDT.
2. The study was done in a non-malaria season. Therefore the prevalence could not reflect what happen all year round.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Specific conclusion

The aim of this study was to determine the efficiency of using malaria RDT as a detection tool for post transfusion malaria in children under five years old. In order to achieve the aim the four objectives had to be alighted.

Objective 1: To determine the prevalence of *Plasmodium* parasitaemia in blood donors.

Conclusion 1

Malaria testing was performed on donors' blood prior to be given out to recipients. The prevalence differs depending on the method used. The prevalence of *Plasmodium* parasitaemia in donor's blood by malaria RDT was 27% and by 7% microscopy.

Objective 2: To determine the prevalence of *Plasmodium* parasitaemia in recipients prior to transfusion.

Conclusion 2

Testing for *Plasmodium* was performed for all recipients enrolled into the study. Prevalence of *plasmodium* parasitaemia in patients prior to transfusion by malaria RDT test method was 34% compared to a prevalence of 13% obtained by microscopy.

Objective 3: To determine the incidence of post transfusion malaria.

Conclusion 3

Incidence was determined in a group of 28 recipients who were found to be parasitaemia negative prior to the start of transfusion. The incidence of post transfusion malaria using PCR was 57%.

Objective 4: To determine the sensitivity of malaria rapid diagnostic test (RDT).

Conclusion 4

Using PCR as the gold standard malaria RDT had 100% sensitivity in detecting *Plasmodium falciparum* infection at post transfusion.

6.2 General conclusion

Malaria rapid diagnostic test (RDT) is a good tool for the detection of post transfusion malaria. Malaria RDT had shown to be more effective than microscopy in *Plasmodium falciparum* malaria detection. In addition, for both the donor blood and in patients prior to transfusion, the prevalence of 27% and 34% respectively by RDT was higher than that microscopy which was 7% and 13% respectively.

6.3 Recommendation

1- It is recommended that the clinical users of blood consider introducing malaria RDT as a test method to screen for malaria at post transfusion.

2- Further studies must be undertaken to compare the different antigens used for malaria RDT in order to identify which is best suitable for post transfusion malaria.

3- There is a need for more training for physicians, so that they do not treat patients who are malaria negative or have not been tested. This could reduce the misuse of ACT thereby preventing early development of resistance.

REFERENCES

Abdul-Aziz, A, R; E, Harris; L. Munyakazi. (2012). risk factors in malaria mortality among children in northern Ghana: a case study at the tamale teaching hospital. *International journal of business and social research, volume 2 (5).*

Acheampong, M. G. Appiah, L. K. Boamponsem, J. N. Boampong and R. Afoakwaah. (2011).The efficacy of rapid diagnostic test (rdt) in diagnosing *Plasmodium Falciparum* malaria in some selected health facilities in the cape coast metropolis of Ghana. J*ournal of Advances in Applied Science Research, volume 2(4), pp348-356.*

Addae-Kyereme, J; Croft, SL; Kendrick, H; Wright, CW. (2001). Anti-plasmodial activities of some Ghanaian plants traditionally used for fever/malaria treatment and of some alkaloids isolated from Pleiocarpa mutica; in vivo antimalarial activity of pleiocarpine, *Journal of Ethnopharmacology, volume 76 (1), pp 99-103. ISSN 0378-874176(1).*

Adeoye, G. O., & Ogbonnaya, C. V. (2014). Relationship of malaria to HBV and HIV status among blood donors in Lagos State. *Nigerian Journal of Parasitology*, *32*(1).

Alam, M. T., de Souza, D. K., Vinayak, S., Griffing, S. M., Poe, A. C., Duah, N. O & Koram, K. A. (2011). Selective sweeps and genetic lineages of Plasmodium falciparum drug-resistant alleles in Ghana. *Journal of Infectious Diseases, volume 203(2), pp220-227.*

Al-Drees, A. M. (2008). Attitude, belief and knowledge about blood donation and transfusion in Saudi population. *Pakistan Journal of Medical Sciences, volume 24(1), pp74.*

Ali, M. S. M., Kadaru, A. A. G. M. Y., & Mustafa, M. S. (2004). Screening blood donors for malaria parasite in Sudan. *Ethiopian Journal of Health Development*, *18*(2), pp 70-74.

Alex Asase, Alfred A. Oteng-Yeboah, George T. Odamtten, Monique S.J. Simmonds. (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. J*ournal of Ethnopharmacology, volume 99 (2005), pp273–279.*

Allain, J. P., Stramer, S. L., Carneiro-Proietti, A. B. F., Martins, M. L., da Silva, S. L., Ribeiro, M & Reesink, H. W. (2009). Transfusion-transmitted infectious diseases. *Biologicals, volume 37(2), pp71-77.*

Allain, J. P., Owusu-Ofori, S., & Bates, I. (2004). Blood transfusion in Sub-Saharan Africa. *Transfusion Alternatives in Transfusion Medicine, volume 6(1), pp16-23.*

Alves-Junior, E. R., Gomes, L. T., Ribatski-Silva, D., Mendes, C. R., Leal-Santos, F. A., Simoes, L. R & Fontes, C. J. (2014). Assumed white blood cell count of 8,000 cells/muL overestimates malaria parasite density in the Brazilian Amazon. *PloS one*, *9*(4).

Akotet, B. M. K., Nkare, C. A., Mbouoronde, O. C., & Mawili-Mboumba, D. P. (2014). *Journal. Malaria Chemoth Cont Elimination, volume 3, pp 125.*

Andrej Trampuz, Matjaz Jereb, Igor Muzlovic, and Rajesh M Prabhu. (2003). Clinical review: Severe malaria. *Critical Care journal, volume 7, pp 315-323 issue 4.*

Andrew D. Haddow mail, Amy J. Schuh, Chadwick Y. Yasuda, Matthew R. Kasper, Vireak Heang, Rekol Huy, Hilda Guzman, Robert B. Tesh, Scott C. Weaver. (2012). Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage. J*ournal PLoS Neglected Tropical Diseases, volume 6(2), pp 477.*

Ansah, E. K., Narh-Bana, S., Affran-Bonful, H., Bart-Plange, C., Cundill, B., Gyapong, M., & Whitty, C. J. (2015). The impact of providing rapid diagnostic malaria tests on fever management in the private retail sector in Ghana: a cluster randomized trial. *Malaria journal*, pp *350*.

Asante, K. P., Abokyi, L., Zandoh, C., Owusu, R., Awini, E., Sulemana, A & Owusu-Agyei, S. (2010). Community perceptions of malaria and malaria treatment behaviour in a rural district of Ghana: implications for artemisinin combination therapy. *Public Health, volume 10(1), pp 409.*

Autino, B., Noris, A., Russo, R., & Castelli, F. (2012). Epidemiology of malaria in endemic areas. *Mediterranean journal of hematology and infectious diseases, volume 4*(1).

Austin N, Adikaibe E, Ethelbert O, Chioma U, Ekene N. (2014). Prevalence and Severity of Malaria Parasitemie among Children Requiring Emergency, Blood transfusion in tertiary hospital in Imo state, Nigeria. *Annals of Medical and Health Science Research; volume 4(4), pp 619-23.*

Bahadur, S., Pujani, M., & Jain, M. (2010). Use of rapid detection tests to prevent transfusion-transmitted malaria in India. *Asian journal of transfusion science, volume 4(2), pp 140.*

Bailey, J. W., Williams, J., Bain, B. J., Parker-Williams, J., & Chiodini, P. L. (2013). Guideline: the laboratory diagnosis of malaria. *British journal of haematology*, *163*(5), pp 573-580.

Banfi G; Salvagno GL; Lippi G. (2007).The role of ethylenediamine tetraacetic acid (EDTA) as in vitro anticoagulant for diagnostic purposes. PubMed, Volume 45(5), pp 565-76.

Bates, I., Owusu-Ofori, A. K., & Parry, C. (2011). Malaria in the blood supply in Africa: Is it a problem? *Vox Sanguinis*, *101*(1), pp 55-55.

Bastiaens, G. J., Bousema, T., & Leslie, T. (2014). Scale-up of malaria rapid diagnostic tests and artemisinin-based combination therapy: challenges and perspectives in sub-Saharan Africa.

Bharti PK, Silawat N, Singh PP, Singh MP, Shukla M, Chand G, Dash AP and Singh N. (2008). The usefulness of a new rapid diagnostic test, the First Response Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *PubMed volume 11(7), pp 126.*

Bhutta, Z. A., Chopra, M., Axelson, H., Berman, P., Boerma, T., Bryce, J & Wardlaw, T. (2010). Countdown to 2015 decade report (2000–10): taking stock of maternal, newborn, and child survival. *The Lancet, volume 375(9730), pp 2032-2044.*

Bloch, E. M., Vermeulen, M., & Murphy, E. (2012). Blood transfusion safety in Africa: a literature review of infectious disease and organizational challenges. *Transfusion medicine reviews, volume 26(2), pp 164-180.*

Boyce, R. M., Muiru, A., Reyes, R., Ntaro, M., Mulogo, E., Matte, M., & Siedner, M. J. (2015). Impact of rapid diagnostic tests for the diagnosis and treatment of malaria at a peripheral health facility in Western Uganda: an interrupted time series analysis. *Malaria Journal, volume 14(1), pp 203.*

Bryce, J., Boschi-Pinto, C., Shibuya, K., Black, R. E., & WHO Child Health Epidemiology Reference Group. (2005). WHO estimates of the causes of death in children. *The Lancet, 365*(9465), pp 1147-1152.

Buabeng, K. O., Duwiejua, M., Dodoo, A. N., Matowe, L. K., & Enlund, H. (2007). Self-reported use of anti-malarial drugs and health facility management of malaria in Ghana. *Malaria journal, volume 6*(1), *pp 85*.

Busch, M. P., Kleinman, S. H., & Nemo, G. J. (2003). Current and emerging infectious risks of blood transfusions. *Jama, volume 289(8), pp 959-962*.

Bruneel, F., Thellier, M., Eloy, O., Mazier, D., Boulard, G., Danis, M., & Bédos, J. P. (2004). Transfusion-transmitted malaria. *Intensive care medicine, volume 30(9), pp 1851-1852*.

Centers for Disease Control and Prevention (CDC). (2005). Fatal bacterial infections associated with platelet transfusions--United States, 2004. *Morbidity and mortality weekly report, volume 54(7), pp 168*.

Chanda, P., Hamainza, B., Moonga, H. B., Chalwe, V., & Pagnoni, F. (2011). Community case management of malaria using ACT and RDT in two districts in Zambia: achieving high adherence to test results using community health workers. *Malaria Journal, volume 10(1), pp 158.*

Chandra, T., Rizvi, S., & Agarwal, D. (2014). Decreasing prevalence of transfusion transmitted infection in Indian scenario. *The Scientific World Journal, 2014*.

Charlotte Monica Mensah and Mohammed Razak Gyasi. (2012). Use of herbal medicine in the management of malaria in the urban-periphery, Ghana. *Biology Agriculture and healthcare journal, Volume 2 (11).*

Chou, M., Kim, S., Khim, N., Chy, S., Sum, S., Dourng, D & Ménard, D. (2012). Performance of "VIKIA Malaria Ag Pf/Pan" (IMACCESS®), a new malaria rapid diagnostic

test for detection of symptomatic malaria infections. *Malaria Journal, volume 11(295), pp 10-1186.*

Choudhury, N. (2010). Transfusion transmitted infections: How many more? *Asian journal of transfusion science, volume 4(2), pp 71.*

ClimaTemp.com. (2015). Kumasi Climate Kumasi temperatures Weather. From www.kumasi.climatemps.com/. Accessed on July 2015.

Cox-Singh, J., Davis, T. M., Lee, K. S., Shamsul, S. S., Matusop, A., Ratnam, S & Singh, B. (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clinical infectious diseases, volume 46(2), pp 165-171.*

Crompton, P. D., Moebius, J., Portugal, S., Waisberg, M., Hart, G., Garver, L. S & Pierce, S. K. (2014). Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annual review of immunology*, volume *32*, pp 157.

Damen, J. G., Barnabas, O., Damulak, D., Ntuhun, B. D., Lugos, M. D., & Nyary, B. (2015). Malaria Parasitaemia in Apparently Healthy Blood Donors in North-Central Nigeria. *Lab Medicine, 46*(1), pp 42-46.

Daniel Eibach, Boubacar Traore, Mourad Bouchrik, Boubacar Coulibaly, Nianégué Coulibaly, Fanta Siby, Guillaume Bonnot, Anne-Lise Bienvenu and Stéphane Picot. (2013). Evaluation of the malaria rapid diagnostic test VIKIA malaria Ag Pf/Pan™ in endemic and non-endemic settings. *Malaria Journal, volume* 12 (188).

De Moraes, C. M., Stanczyk, N. M., Betz, H. S., Pulido, H., Sim, D. G., Read, A. F., & Mescher, M. C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences, volume 111(30), pp 11079-11084.*

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J & White, N. J. (2009). Artemisinin resistance in Plasmodium falciparum malaria. *New England Journal of Medicine, volume 361(5), pp 455-467.*

Eder, A. F., Kennedy, J. M., Dy, B. A., Notari, E. P., Weiss, J. W., Fang, C. T & Benjamin, R. J. (2007). Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). *Transfusion, volume 47(7), pp 1134-1142.*

Eisele, T. P., Larsen, D. A., Walker, N., Cibulskis, R. E., Yukich, J. O., Zikusooka, C. M., & Steketee, R. W. (2012). Estimates of child deaths prevented from malaria prevention scale-up in Africa 2001–2010. *Malaria Journal, volume 11(1), pp 93.*

Elphinstone, R. E., Higgins, S. J., & Kain, K. C. (2014). Prevention of Malaria in Travelers: Bite Avoidance and Chemoprophylactic Measures. *Current Treatment Options in Infectious Diseases, volume 6(1), pp 47-57.*

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P. M., Shargie, E. B., Ejigsemahu, Y & Richards, F. O. (2008). Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malaria journal, volume 7(1), pp 118.*

Erhabor Osaro , Adias Teddy Charles. (2011).The challenges of meeting the blood transfusion requirements in Sub-Saharan Africa: the need for the development of alternatives to allogenic blood. *Journal of Blood Medicine, volume 2, pp 7–21.*

Freedman, D. O. (2008). Malaria prevention in short-term travelers. *New England Journal of Medicine, volume 359(6), pp 603-612.*

Freimanis, G., Sedegah, M., Owusu-Ofori, S., Kumar, S., & Allain, J. P. (2013). Investigating the prevalence of transfusion transmission of Plasmodium within a hyperendemic blood donation system. *Transfusion, 53*(7), pp 1429-1441.

Gagandeep Singh and Rakesh Sehgal. (2010). Transfusion-transmitted parasitic infections. *Asian Journal Transfusion Science. 2010 volume 4(2), pp 73–77.*

Gatti, M. Gramegna, Z. Bisoffi, A. Raglio1, M. Gulletta, C. Klersy, A. Bruno, R. Maserati, S. Madama, M. Scaglia and the Gispi study Group Annals of Tropical Medicine & Parasitology. (2007). A comparison of three diagnostic techniques for malaria rapid diagnostic test (NOWH Malaria), PCR and microscopy. *Annals of tropical medicine and parasitology, Volume 101 (3), pp 195-204.*

Gay-Andrieu, F., Adehossi, E., Lacroix, V., Gagara, M., & Ibrahim, M. L. (2014). Epidemiological clinical and biological features of malaria among children in Niamey Niger. *Malaria Journal*, volume *4* (9).

Gerstl, S., Dunkley, S., Mukhtar, A., De Smet, M., Baker, S., & Maikere, J. (2010). Assessment of two malaria rapid diagnostic tests in children under five years of age, with follow-up of false-positive pLDH test results, in a hyperendemic falciparum malaria area. *Sierra Leone. Malaria Journal, volume 9, pp 28.*

Gillet, P., Ngoyi, D. M., Lukuka, A., Kande, V., Atua, B., van Griensven, J & Lejon, V. (2013). False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human african trypanosomiasis.

National Malaria Control Programme. (2014). Ghana records over 11 million cases of OPD malaria. Ghana national agency (GNA) from www.ghananewsagency.org last accessed Jun 28, 2015.

Gutman, J., Mwandama, D., Wiegand, R. E., Abdallah, J., Iriemenam, N. C., Shi, Y. P & Skarbinski, J. (2015). In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi. *Malaria Journal, volume 14(1), pp 197.*

Greenwood, B., & Mutabingwa, T. (2002). Malaria in 2002. *Nature, volume 415(6872), pp 670-672.*

Greenwood, B. (2010). Anti-malarial drugs and the prevention of malaria in the population of malaria endemic areas. *Malaria Journal, volume 9, pp 2.*

Goodnough, L. T. (2003). Risks of blood transfusion. *Critical care medicine, volume 31*(12), pp 678-S686.

Hendriksen, I. C., White, L. J., Veenemans, J., Mtove, G., Woodrow, C., Amos, B & Dondorp, A. M. (2013). Defining falciparum-malaria-attributable severe febrile illness in moderate-to-high transmission settings on the basis of plasma PfHRP2 concentration. *Journal of Infectious Diseases*, *207*(2), pp 351-361.

Hendriksen, I. C., Mwanga-Amumpaire, J., Von Seidlein, L., Mtove, G., White, L. J., Olaosebikan, R & Dondorp, A. M. (2012). Diagnosing severe falciparum malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. *PLoS medicine*, *9*(8), pp 1255.

Hillyer, C. D., Josephson, C. D., Blajchman, M. A., Vostal, J. G., Epstein, J. S., & Goodman, J. L. (2003). Bacterial Contamination of Blood Components: Risks, Strategies, and Regulation Joint ASH and AABB Educational Session in Transfusion Medicine. *ASH Education Program Book, volume (1), pp 575-589*.

Hodgson, S. H., Douglas, A. D., Edwards, N. J., Kimani, D., Elias, S. C., Chang, M & Murphy, S. C. (2015). Increased sample volume and use of quantitative reverse-transcription PCR can improve prediction of liver-to-blood inoculum size in controlled human malaria infection studies. *Malaria journal, volume 14(1), pp 1-9.*

Hopkins, H., González, I. J., Polley, S. D., Angutoko, P., Ategeka, J., Asiimwe, C & Bell, D. (2013). Highly sensitive detection of malaria parasitaemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. *Journal of Infectious Diseases*, *208*(4), pp 645-652.

Hunter, N., Foster, J., Chong, A., McCutcheon, S., Parnham, D., Eaton, S & Houston, F. (2002). Transmission of prion diseases by blood transfusion. *Journal of General Virology, volume 83(11), pp 2897-2905.*

Idro, R., Jenkins, N. E., & Newton, C. R. (2005). Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *The Lancet Neurology, volume 4(12), pp 827-840.*

João C K Dos-Santos, Rodrigo N Angerami, Catarina M S Castiñeiras, Stefanie C P Lopes, Letusa Albrecht, Márcia T Garcia, Carlos E Levy, Maria L Moretti, Marcus V G Lacerda and Fabio T M Costa. (2014). Imported malaria in a non-endemic area: the experience of the university of Campinas hospital in the Brazilian Southeast. Malaria. *Journal, volume 13 (280) pp 1475-2875.*

Jobert Richie N Nansseu, Jean Jacques N Noubiap, Shalom Tchokfe Ndoula, Albert Frank M Zeh and Chavely Gwladys Monamele. (2013).What is the best strategy for the prevention of transfusion-transmitted malaria in sub-Saharan African countries where malaria in endemic? *Malaria Journal, volume 12 (465), pp 1475-2875.*

Joel C. Mouatcho and J. P. Dean Goldring. (2013). Malaria rapid diagnostic tests: challenges and prospects. *Journal of Medical Microbiology, volume 62, pp 1491–1505.*

Johnston, S. P., Pieniazek, N. J., Xayavong, M. V., Slemenda, S. B., Wilkins, P. P., & da Silva, A. J. (2006). PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of clinical microbiology, volume 44(3), pp 1087-1089.*

Kátia Luz Torres, Mônica Nascimento dos Santos Moresco, Luciane Rodrigues Sales, Josilene da Silva Abranches, Márcia Almeida Araújo Alexandre and Adriana Malheiro. (2014). Transfusion-transmitted malaria in endemic zone: epidemiological profile of blood donors at the Fundação HEMOAM and use of rapid diagnostic tests for malaria screening in Manaus. *Rev. Bras. Hematol. Hemoter. volume.36 no.4, pp* 269–274.

Kakkilaya, B. S. (1990). Are We Treating Malaria? *World malaria, volume 3(1990), pp 3.*

Keating, J., Finn, T. P., Eisele, T. P., Dery, G., Biney, E., Kêdoté, M & Yukich, J. O (2014). An assessment of malaria diagnostic capacity and quality in Ghana and the Republic of Benin. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 108(10), pp 662-669.*

Kelly, M., Su, C. Y., Schaber, C., Crowley, J. R., Hsu, F. F., Carlson, J. R., & Odom, A. R. (2015). Malaria parasites produce volatile mosquito attractants. *mBio*, *6*(2), e00235-15.
Kitchen, A. D., & Chiodini, P. L. (2006). Malaria and blood transfusion. *Vox sanguinis, volume 90(2), pp 77-84*.

Kitchen, A., Mijovic, A., & Hewitt, P. (2005). Transfusion-transmitted malaria: current donor selection guidelines are not sufficient. *Vox sanguinis*, *88*(3), pp 200-201.

Kobbe, R., Klein, P., Adjei, S., Amemasor, S., Thompson, W. N., Heidemann, H & May, J. (2008). A randomized trial on effectiveness of artemether-lumefantrine versus artesunate plus amodiaquine for unsupervised treatment of uncomplicated Plasmodium falciparum malaria in Ghanaian children. *Malaria Journal, volume 7, pp.261.*

Kochar, D. K., Tanwar, G. S., Khatri, P. C., Kochar, S. K., Sengar, G. S., Gupta, A & Das, A. (2010). Clinical features of children hospitalized with malaria—a study from Bikaner, northwest India. *The American journal of tropical medicine and hygiene, volume 83(5), pp 981-989.*

Konaté, A. T., Yaro, J. B., Ouédraogo, A. Z., Diarra, A., Gansané, A., Soulama, I & Diallo, D. A. (2011). Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial. *PLoS Med*, *8*(2).

Laban, N. M., Kobayashi, T., Hamapumbu, H., Sullivan, D., Mharakurwa, S., Thuma, P. E & Moss, W. J. (2015). Comparison of a PfHRP2-based rapid diagnostic test and PCR for malaria in a low prevalence setting in rural southern Zambia: implications for elimination. *Malaria journal*, *14*(1), pp 25.

Li, Y., Kumar, N., Gopalakrishnan, A., Ginocchio, C., Manji, R., Bythrow, M, & Kong, H. (2013). Detection and species identification of malaria parasites by isothermal tHDA amplification directly from human blood without sample preparation. *The Journal of Molecular Diagnostics, volume 15(5), pp 634-641.*

Lilit Garibyan and Nidhi Avasshia. (2013). Polymerase chain reaction. *Journal of Invest Dermatology, volume 133(3) pp 6.*

Lund, T. C., Hume, H., Allain, J. P., McCullough, J., & Dzik, W. (2013). The blood supply in Sub-Saharan Africa: Needs, challenges, and solutions. *Transfusion and Apheresis Science*, *49*(3), pp 416-421.

Mackintosh, C. L., Beeson, J. G., & Marsh, K. (2004). Clinical features and pathogenesis of severe malaria. *Trends in parasitology*, *20*(12), pp 597-603.

Madhad, V. J., & Sentheil, K. P. (2014). The Rapid & Non-Enzymatic isolation of DNA from the Human peripheral whole blood suitable for Genotyping. *European Journal of Biotechnology and Bioscience, 1*(3), pp 01-16.

Mali, S., Kachur, S. P., & Arguin, P. M. (2012). Malaria surveillance—United States, 2010. *MMWR Surveill Summ, volume 61(2), pp 1.*

Manyando, C., Njunju, E. M., Chileshe, J., Siziya, S., & Shiff, C. (2014). Rapid diagnostic tests for malaria and health workers' adherence to test results at health facilities in Zambia. *Malaria Journal*, *13*(166), pp 10-1186.

Mark E. Brecher and Shauna N. Hay. (2005). Bacterial Contamination of Blood Components Department of Pathology and Laboratory Medicine, University of North Carolina. *Journal of Clinical Microbiololy Review, volume 18(1), pp 195–204.*

Marc C Tahita, Halidou Tinto, Joris Menten, Jean-Bosco Ouedraogo, Robert T Guiguemde,Jean Pierre van Geertruyden, Annette Erhart and Umberto D'Alessandro. (2013). Clinical signs and symptoms cannot reliably predict *Plasmodium falciparum* malaria infection in pregnant women living in an area of high seasonal transmission. *Malaria Journal, volume 12 (464).*

Masanja, I. M., Selemani, M., Amuri, B., Kajungu, D., Khatib, R., Kachur, S. P., & Skarbinski, J. (2012). Increased use of malaria rapid diagnostic tests improves targeting of anti-malarial treatment in rural Tanzania: implications for nationwide rollout of malaria rapid diagnostic tests. *Malaria Journal, volume 11(1), pp 221.*

Mavenyengwa, R. T., Mukesi, M., Chipare, I., & Shoombe, E. (2014). Prevalence of human immunodeficiency virus, syphilis, hepatitis B and C in blood donations in Namibia. *BMC public health, 14*(1), pp 424.

Mbakilwa, H., Manga, C., Kibona, S., Mtei, F., Meta, J., Shoo, A & Reyburn, H. (2012). Quality of malaria microscopy in 12 district hospital laboratories in Tanzania. *Pathogens and global health*, *106*(6), pp 330-334.

Mens, P., Spieker, N., Omar, S., Heijnen, M., Schallig, H. D. F. H., & Kager, P. A. (2007). Is molecular biology the best alternative for diagnosis of malaria to microscopy? A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania. *Tropical Medicine & International Health*, *volume 12(2), pp 238-244.*

Micheal L Wilson MD. (2013). Laboratory diagnosis of malaria. *Arch Pathol Lab Med—Volume 137(6), pp 805-11.*

Mudanyali, O., Dimitrov, S., Sikora, U., Padmanabhan, S., Navruz, I., & Ozcan, A. (2012). Integrated rapid-diagnostic-test reader platform on a cellphone. *Lab on a Chip*, *12*(15), pp 2678-2686.

Mukadi, P., Gillet, P., Lukuka, A., Atua, B., Kahodi, S., Lokombe, J & Jacobs, J. (2011). External quality assessment of malaria microscopy in the Democratic Republic of the Congo. *Malaria Journal*, volume *10*, pp 308.

Musso, T Nhan, E Robin, C Roche, D Bierlaire, K Zisou, A Shan Yan, V M Cao-Lormeau, J Broult. (2014). Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia. From *Eurosurveillance journal, Volume 19 (14).*

Mungai, M., Tegtmeier, G., Chamberland, M., & Parise, M. (2001). Transfusion-transmitted malaria in the United States from 1963 through 1999. *New England Journal of Medicine, volume 344(26), pp 1973-1978.*

Muntaka, S., & Opoku-Okrah, C. (2013). The Prevalence of Malaria Parasitaemia and Predisposition of ABO Blood Groups to Plasmodium falciparum Malaria among Blood Donors at a Ghanaian Hospital. *Assumption University Journal of Technology*, *16*, pp 255-260.

Nabajyoti Choudhury. (2010). Transfusion transmitted infections: How many more? *Asian Journal Transfus Science, volume 4(2), pp 71–72.*

Nahom Fessehaye, Durgadas Nail, Tesfay Fessehaye. (2011). Transfusion transmitted. infections – A retrospective analysis from the National Blood Transfusion Service in Neergheen-Bhujun, V. S. (2013). Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. *BioMed research international, 2013.*

Newman, R. D. (2012). World Malaria Report 2011.

Noubouossie, D., Tagny, C. T., Same-Ekobo, A., & Mbanya, D. (2012). Asymptomatic carriage of malaria parasites in blood donors in Yaoundé. *Transfusion Medicine*, *22*(1), pp 63-67.

Nyarko, S. H., & Cobblah, A. (2014). Sociodemographic Determinants of Malaria among Under-Five Children in Ghana. *Malaria research and treatment*, *2014*.

Obi, E., Agbasi, P., Orisakwe, O., & Nriagu, J. (2015). Cadmium Levels in Maternal and Cord Blood and Anthropometric Parameters of Newborns in Nnewi, South-Eastern Nigeria. *The FASEB Journal*, *29*(1 Supplement), pp 1023-6.

O'Brien, S. F., Delage, G., Seed, C. R., Pillonel, J., Fabra, C. C., Davison, K & Leiby, D. A. (2015). The Epidemiology of Imported Malaria and Transfusion Policy in 5 Nonendemic Countries. *Transfusion medicine reviews*.

Orla Geoghegan & Imogen Clarke . (2014). Malaria clinical features and prevention. *Journal of Clinical Pharmacist, Volume 6 (3), p 57.*

Ouattara, A., Doumbo, S., Saye, R., Beavogui, A. H., Traoré, B., Djimdé, A & Thera, M. A. (2011). Use of a pLDH-based dipstick in the diagnostic and therapeutic follow-up of malaria patients in Mali. *Malaria Journal, volume 10, pp 345.*

Owusu-Ofori, A. K., Parry, C., & Bates, I. (2010). Transfusion-transmitted malaria in countries where malaria is endemic: a review of the literature from sub-Saharan Africa. *Clinical infectious diseases, volume 51(10), pp 1192-1198.*

Owusu-Ofori, A. (2012). *Transfusion-transmitted malaria and bacterial infections in a malaria endemic region* (Doctoral dissertation, University of Liverpool).

Owusu-Ofori, Martha Betson, Christopher M. Parry, J. Russell Stothard, and Imelda Bates. (2013). Transfusion-Transmitted Malaria in Ghana. *Journal of Clinical Infectious Diseases, pp 1735-1741.*

Paglia, M. G., Vairo, F., Bevilacqua, N., Ghirga, P., Narciso, P., Severini, C., & Nicastri, E. (2012). Molecular diagnosis and species identification of imported malaria in returning travellers in Italy. *Diagnostic microbiology and infectious disease*, *72*(2), pp 175-180.

President's Malaria Initiative. (2013a). President's malaria initiative Uganda malaria operational plan FY 2013. *USAID, US Department of Health and Human Services and the Centers for Disease Control. Washington, DC*.

President's Malaria Initiative. (2013b). Report of the Ghana Urban malaria study January 2013. From www.jsi.com/Independent/Docs/GhanaUrbanMalariaStudy.pdf Accessed on July 2015.

Prugnolle, F., Durand, P., Neel, C., Ollomo, B., Ayala, F. J., Arnathau, C & Renaud, F. (2010). African great apes are natural hosts of multiple related malaria species, including

Plasmodium falciparum. *Proceedings of the National Academy of Sciences, volume 107(4), pp 1458-1463.*

Regan, F., & Taylor, C. (2002). Recent developments: Blood transfusion medicine. *British Medical Journal*, volume 325(7356), pp 143.

Reyburn, H., Mbakilwa, H., Mwangi, R., Mwerinde, O., Olomi, R., Drakeley, C., & Whitty, C. J. (2007). Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *Biomedical journal, volume 334(7590), pp 403.*

Sachs, J., & Malaney, P. (2002). The economic and social burden of malaria. *Nature, volume 415(6872), pp 680-685.*

Shekalaghe, S., Cancino, M., Mavere, C., Juma, O., Mohammed, A., Abdulla, S., & Ferro, S. (2013). Clinical performance of an automated reader in interpreting malaria rapid diagnostic tests in Tanzania. *Malaria Journal, 12*(141), pp 10-1186.

Shimoyama, R. (2008). [Transfusion-transmitted diseases]. *[Hokkaido igaku zasshi] The Hokkaido journal of medical science, volume 83(1), pp 5-21.*

Singh, G., & Sehgal, R. (2010). Transfusion-transmitted parasitic infections. *Asian journal of transfusion science, volume 4(2), pp 73.*

Smita Mahapatra. (2015). Prevalence of transfusion transmitted infection giving importance to HIV in screening of health blood donors and the challenges ahead. *Journal of Clinical Research HIV/AIDS, volume 2(1), pp 1013.*

Tagny, C. T., Owusu-Ofori, S., Mbanya, D., & Deneys, V. (2010). The blood donor in Sub-Saharan Africa: a review. *Transfusion Medicine, volume 20(1), pp 1-10.*

Tagny, C. T., Mbanya, D., Tapko, J. B., & Lefrère, J. J. (2008). Blood safety in Sub-Saharan Africa: a multi-factorial problem. *Transfusion, volume 48(6), pp 1256-1261.*

Tayou, T. C., Mbanya, D., Garraud, O., & Lefrère, J. J. (2007). [Blood safety: malaria and blood donation in Africa]. *Transfusion clinique et biologique: journal de la Societe francaise de transfusion sanguine, volume 14*(5), *pp 481-486.*

Tek, F. B., Dempster, A. G., & Kale, I. (2009). Computer vision for microscopy diagnosis of malaria. *Malaria Journal, volume 8(1), pp 153.*

Tembo, D. L. (2013). *Antigen expression and host-parasite interactions of Plasmodium falciparum infections in Malawian paediatric patients* (Doctoral dissertation, Liverpool School of Tropical Medicine).

Transfusion Today. (2012). Goals and achievement of the Kumasi Blood centre, Ghana, September 2012. Number 92. From www.trec.eu/highlights/documents/KumasiBloodCentre.pdf Accessed on July 2015.

Vamvakas, E. C., & Blajchman, M. A. (2009). Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood, volume 113(15), pp 3406-3417.*

Weekley, C., & Smith, D. S. (2013). Malaria: the clinical basics. *Global Health Education Consortium.*

William Stauffer and Philip R. Fischer. (2003). Diagnosis and Treatment of Malaria in Children 2003. *Journal of Clinical Infectious Diseases volume 37(10), pp 1340-1348.*

Yang, S., & Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *The Lancet infectious diseases, volume 4(6), pp 337-348.*

World Health Organization (WHO). 2014. World malaria report 2014. From www.who.int/malaria/...malaria_report_2014/wmr-2014-no-profiles.pdf . Accessed on July 2015.

World Health Organization (WHO). 2015. Guidelines for the treatment of malaria, third edition. From *apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf* Accessed on July 2015.

Zhao, J., Lama, M., Korenromp, E., Aylward, P., Shargie, E., Filler, S & Atun, R. (2012). Adoption of rapid diagnostic tests for the diagnosis of malaria, a preliminary analysis of the global fund program data, 2005 to 2010. *PloS one, volume 7(8), pp 435-49.*

APPENDICES

Appendix 1: Materials

Test methods	Materials
Samples collection	Sterile 2ml syringes 70% Alcohol Plaster Cotton Gloves Tourniquet Sterile lancet EDTA tube Freezer -20°C Rack
Malaria RDT (made to detect Pf HRP2 and Pan LDH)	Malaria RDT kit Gloves Micropipette Timer
Microscopy	Microscopy slides Gloves Rack Microscope Timer
PCR	Pipettes 10, 20, 100, 200 and 1000 ml Racks Centrifuge Cryo-box Thermal cycler Electrophoresis tank Casting tray Comb PCR' tubes RTA receiver tubes Reaction tubes Freezer (-20°C) Timer

Appendix 2: Informed consent form

Title of the research: Determining the efficiency of malaria rapid diagnostic test to diagnose post transfusion malaria in children under five years old at Komfo Anokye Teaching Hospital
Name(s) and affiliation(s) of researcher(s) of applicant(s): Miss Djeneba BOUARE.
Introduction:
I am a student of KNUST. I am carrying a research work in KATH and wish to explain this study to you and to obtain your consent to participate in this study.
Purpose(s) of research:
The purpose of this research is to determine the feasibility of malaria rapid diagnostic tests (RDT) to detect post transfusion transmitted malaria.
Selection of participants:
This study involves children under 5 years old who receive blood transfusion.
Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:
Your child/your relative is about to receive a blood/blood product transfusion. This blood has been investigated for HIV, Hepatitis B, C and syphilis and is considered safe. However malaria parasites have not been looked for because it is not routinely done in this country. What this study seeks to do is to look for malaria parasites in the received blood bag and in the child blood.
If you agree to be enrolled into this study, before the transfusion is started, we will take one millilitre of your blood and test it for malaria parasites.
One day after the transfusion another one millilitre of blood will be collected and test for malaria parasites. Any malaria symptoms will be noted. On day three and five the same process will be done as on day one.
Risk(s):
There is the possibility of feeling pain when we are taking your blood and the place from where we take the blood may be bruised.
Benefit(s):
The immediate benefit for you in this study is that you will have direct access to a medical team in the event that you develop any symptoms and at no extra expense to you.
The goal of this research is to find out if there are malaria parasites in the donated blood and if the malaria rapid diagnostic tests are able to determine any post transfusion transmitted malaria. What we find could be able to help us provide prompt malaria treatment as soon as malaria

parasites are found in your blood and that will avoid any delay for treatment hence avoiding any malaria complications.

Confidentiality:

All the information collected from you in this study will be well kept by us and not given to anybody. However, the people who approved this study may sometimes carry out inspections, so if that happens in this study, we will show them the information collected but without your personal details.

Your name will be initially recorded to be able to trace you in case of any eventuality. However all names will be deleted from our records immediately the study ends.

Your name will not be published.

Voluntariness:

Your participation is entirely voluntary and can decide to participate in this study or not.

Alternatives to participation:

If you chose not to participate in this study, it will not affect the way you will be treated in this hospital.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:

You may choose to withdraw from this study at any time. Please note that some of the information that has been obtained about you before you chose to withdraw may have been used in reports and publications and cannot be removed anymore. However we promise to comply with your wishes as much as is practicable.

Dissemination of results:

Results of this study will be shared with the management of the hospital and the ministry of health. It will also be published in international journals.

Study contact person: In case you have any questions, difficulties, or emergencies, you can contact Miss Djeneba BOUARE on 026 59 89 104 or my supervisor Dr Alex Owusu-Ofori, Directorate of Diagnostics, KATH, Kumasi; Phone number 0244 605 543.

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: _____ SIGNATURE: _____

NAME: _____

Statement of person giving consent:

I have read the description of the research or have had it translated into language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' SIGNATURE (if applicable): _____

WITNESS' NAME (if applicable): _____

Tel number: _____

Address:_____

_____

_____

_____

_____

Appendix 3: Case report form

Pre-transfusion sampling and data collection

Recipient's name: …… Address:…….

City: ……. Age: …… mm/dd/yy

Sex: ……

Code attributed to recipient: ……..

Code attributed to blood bag: ……..

Admission's reasons: ……

Anti-malarial treatment given before transfusion; ……

Any malaria diagnosis done before study: ……

If yes: Results: ……..

Tests used for diagnosis: ……

Date of sampling: ……

Date of sample tested: ……..

Results of recipient's diagnosis for malaria parasites: RDT: ……

Microscopy: …….

PCR: ……..

Diagnosis of blood bag for malaria parasites: RDT: …….

Microscopy: ……..

PCR: …….

Date (s) of blood transfusion; ……..

Post transfusion sampling and data collection

Treatment given: ……

If yes name (s) of drug (s): …….

Any other malaria diagnosis done on the recipient: ……

Conditions preventing sample collection: …….

Date of sampling: ……

Symptoms encountered: ……

Date of sample tested: ……..

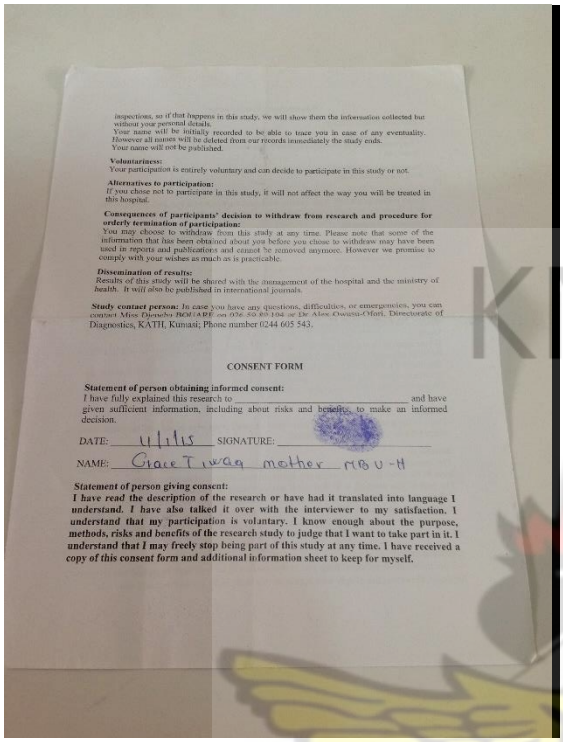
Results of recipient's diagnosis for malaria parasites: RDT: ……

Microscopy: …….

PCR: …….

Appendix 4: Examples of approved consent forms with signature (B) and finger print (A)

A



inspections, so if that happens in this study, we will show them the information collected but without your personal details.
Your name will be initially recorded to be able to trace you in case of any eventuality. However all names will be deleted from our records immediately the study ends.
Your name will not be published.

Voluntariness:
Your participation is entirely voluntary and can decide to participate in this study or not.

Alternatives to participation:
If you chose not to participate in this study, it will not affect the way you will be treated in this hospital.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:
You may choose to withdraw from this study at any time. Please note that some of the information that has been obtained about you before you chose to withdraw may have been used in reports and publications and cannot be removed anymore. However we promise to comply with your wishes as much as is practicable.

Dissemination of results:
Results of this study will be shared with the management of the hospital and the ministry of health. It will also be published in international journals.

Study contact person: In case you have any questions, difficulties, or emergencies, you can contact Miss Djaneba BOUARE on 026 50 85 184 or Dr Alex Owusu-Ofori, Directorate of Diagnostics, KATH, Kumasi; Phone number 0244 605 543.

CONSENT FORM

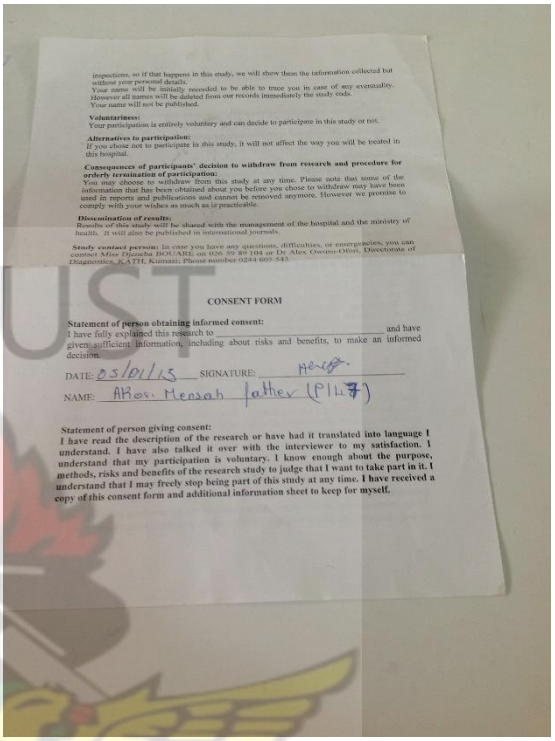
Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: 4/1/15 SIGNATURE: _____

NAME: Grace Tiwaa mother MBU-H

Statement of person giving consent:
I have read the description of the research or have had it translated into language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

B



inspections, so if that happens in this study, we will show them the information collected but without your personal details.
Your name will be initially recorded to be able to trace you in case of any eventuality. However all names will be deleted from our records immediately the study ends.
Your name will not be published.

Voluntariness:
Your participation is entirely voluntary and can decide to participate in this study or not.

Alternatives to participation:
If you chose not to participate in this study, it will not affect the way you will be treated in this hospital.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:
You may choose to withdraw from this study at any time. Please note that some of the information that has been obtained about you before you chose to withdraw may have been used in reports and publications and cannot be removed anymore. However we promise to comply with your wishes as much as is practicable.

Dissemination of results:
Results of this study will be shared with the management of the hospital and the ministry of health. It will also be published in international journals.

Study contact person: In case you have any questions, difficulties, or emergencies, you can contact Miss Djaneba BOUARE on 026 50 85 184 or Dr Alex Owusu-Ofori, Directorate of Diagnostics, KATH, Kumasi; Phone number 0244 605 543.

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: 05/01/15 SIGNATURE: _____

NAME: Alex. Mensah father (PIL87)

Statement of person giving consent:
I have read the description of the research or have had it translated into language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

Appendix 5: Giemsa solution used for microscopy slides

Thick and thin films' preparation for malaria microscopy

Thick and thin blood films were prepared from blood collected from the vein or third finger or foot from the recipient or blood bag into EDTA tubes. A pipette was used to transfer two distinct drops of blood samples from EDTA tubes unto the glass slides. Thick and thin films were prepared unto the same slide. Slides were labelled appropriately with the recipient's code.

Thin film

A drop of blood was placed in the middle of glass slide. Another glass slide with clean and smooth end was placed in contact with the drop of blood forming an angle of 45° to enable the blood to spread along the edge of the second glass slide which acts as the spreader. The spreader was pushed forward to the end while maintaining the angle. This ensures that the blood is spread thinly.

The slide was air dried and fixed by dipping into 100% methanol for about 10 seconds. After it was air dry. The smear was then stained with a newly prepared 5% Giemsa stain solution for 45 minutes and washed with clean the distilled water.

The slide was then placed on a drying rack and allowed to dry at room temperature.

Thick film

A drop of blood was placed on one side of the glass slide. Using the corner of another clean and smooth glass slide, the drop of blood was spread in a circular way to ensure the spread of the blood with moderate thickness. The blood was air dried without being fix in methanol. The smear was then stained with a newly prepared 5% Giemsa stain solution for 45 minutes and washed with clean distilled water.

The slide was then placed on a drying rack and allowed to air dry at room temperature.

The stained blood smears were examined with 100X oil immersion objective for both think and thin films.

Thin film fixation in methanol

5% Giemsa stain preparation

Slides' staining with 5% Giemsa

Slides observation under microscope using 100X