SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

SCREENING FOR PLASMODIUM FALCIPARUM MALARIA USING MRD TEST IN A HIGH TRANSMISSION AREA OF GHANA: KINTAMPO DISTRICT.

BY

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THIS DISSSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT FOR THE AWARD OF MSC CLINICAL TRIALS DEGREE

JULY 2013

DECLARATION

I, Dennis Boateng, do hereby declare that this dissertation is an original research work carried out by me using secondary data collected from the Kintampo North Municipality of Ghana from June 2005 and May 2006. I have not presented it either in part or wholly anywhere else for any reward or publication whatsoever.

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DEDICATION

This dissertation is dedicated to my late mother Alice Adjei who will always be a part of me.

ACKNOWLEDGMENT

I wish to acknowledge with thanks the financial support received from the INDEPTH NETWORK and the Kintampo Health Research Centre without which fulfillment of this ambition would not have been possible.

My greatest appreciation and gratitude go to the staff and Management of School of Public Health (SPH), University of Ghana, with particular reference to my academic supervisor and Mentor, Professor Fred Binka, Vice Chancellor of University of Health and Allied Sciences (UHAS) in the Volta Region under whose watchful eyes a dream has come true. Also appreciation goes to the head of Epidemiology Department Dr. Patricia Akweongo and course coordinator, Mrs. Regina Afari Boateng who relayed relevant information to carry out this work successfully.

Many thanks and appreciation also go to the Director of Kintampo Health Research Centre Dr. Seth Owusu-Agyei, Dr. Kwaku Poku Asante, Principal investigator and Emmanuel Mahama, statistician under whose supervision data and other required tools were obtained.

My acknowledgement would be incomplete without paying a very special tribute to all the staff of Kintampo Health Research Centre who made it possible for me to obtain the information and data to accomplish my research work and secondary analysis.

To you all, I extend my best wishes and may the good lord richly reward you.

Summary

Background

Malaria has a high burden in sub-Saharan Africa which is caused by *Plasmodium falciparum.* Delayed or inaccurate diagnosis of malaria could lead to fatal consequences in patients seeking medical care for malaria. Malaria Rapid Diagnostic Tests (mRDT) provide an alternative in the absence of malaria microscopy to provide prompt and accurate diagnosis and is used to complement clinical diagnosis of individuals who report at health facilities with unspecific symptoms. Low sensitivity and specificity of mRDTs may lead to under-diagnosis or over diagnosis of malaria. Hence there is the need to confirm the validity of the mRDT in specific malaria transmission zones. The aim of this study was to assess the sensitivity and specificity of mRDT in the Kintampo Municipality of Ghana.

Methods

A secondary data analysis was done using a cross sectional study design data from the Kintampo Malarial Drug Trial conducted during the period of June 2005 to May 2006 to assess the efficacy of Artemisinin combination therapy. Cleaned data were analyzed using Stata 11.0 (STATACORP, TX.). Demographic characteristics of study children that were categorical in nature were summarized as proportions, while quantitative variables such as hemoglobin concentration was summarized as means based on their distributions. Sensitivity and specificity analysis of the mRDT was explored using malaria microscopy as the gold standard and Receiver Operating Characteristic (ROC) curve was used to determine the ability of the RDT test to correctly classify patients with or without malaria parasitaemia. Univariate logistic regression

was used to explore crude relationships between socio-demographic and potential confounding variables with clinical malaria. Clinical malaria was defined as reported or measured fever with malaria parasite counts >0. All statistically significant variables identified from the univariate analysis were adjusted for in a multivariate logistic regression model.

Results:

A total of nine hundred and thirty seven (937) children between 0- 10 years who were screened in the primary study were analyzed in this study. About 99% (923/937) of the children enrolled had both malaria parasitaemia and mRDT done. Using microscopy as the gold standard, the overall sensitivity and specificity of the mRDT used were 93.2% (95% CI: 90.7-95.2) and 83.0% (95% CI: 78.9-86.5) with a positive predictive value of (PPV) 88.1% (95% CI: 85.1- 90.6) and negative predictive value (NPV) of 90.1% (95% CI: 86.5-92.9) respectively. The risk of clinical malaria was 1.45 times more likely among children analyzed in this study with abnormal liver function test compared to those with normal liver function test [aOR 1.45 (95% CI:1.05-2.01), p=0.03] after adjusting for age and sex in a multivariate logistic regression model.

Conclusion

This mRDT maintains its high sensitivity and specificity among patients in the forest-savanna transitional zone of Ghana. The Malaria Rapid Diagnostic Test may be provided to peripheral public health facilities that do not have trained laboratory technicians, electricity and laboratory to appropriately diagnose malaria.

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LIST OF ACRONYMS

ACPR	Adequate Clinical Parasitological Response
ACT	Artemisinin-based Combination Therapy
GHS	Ghana Health Service
HRP-2	Histidine Rich Protein 2
IPD	In-Patient Department
LFT	Liver Function Test
KMDT	Kintampo Malaria Drug Trial
MRDT	Malaria Rapid Diagnostic Test
NPV	Negative Predictive Value
OPD	Out-Patient Department
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
RCT	Randomized Control Trial
RDT	Rapid Diagnostic Test
RFT	Renal Function Test
ROC	Receiver Operating Characteristic

SOP	Standard Operating Procedure
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

In sub-Saharan Africa malaria accounts for the majority of deaths and contributes to the global burden of infectious disease (Kebede, Duales & Alemu, 2010). Delayed and inaccurate measurement resulting from malaria diagnosis in children could lead to fatal consequences including death and hospitalization caused by *Plasmodium species. Plasmodium falciparum* is the most predominant species in sub-Sahara Africa and leads to severe diseases such as severe anemia, cerebral malaria and intravascular hemolysis (Davenport, Hittner, Were, Ong'echa & Perkins, 2012). Currently, malaria is estimated to cause 216 million clinical episodes and 655,000 deaths with 91% of estimated deaths in the African Region (CDC, 2012) with approximately 100 million children aged under five years live in areas where malaria transmission occurs (Rowe et al., 2006).The direct cost of malaria including illness, treatment, and premature death has been estimated to be at least US$ 12 billion per year (CDC, 2011; WHO, 2010).

Malaria can be diagnosed presumptively using patient clinical history (presumptive diagnosis), and/or identification of malaria parasites using malaria microscopy or malaria rapid diagnostic test (mRDTs) (Ansumana et al., 2013). The World Health Organization (WHO) currently recommends confirmed malaria diagnosis with malaria microscopy or mRDTs prior to treatment with Artemisinin-based Combination Therapy (ACTs) (WHO, 2010). However, in malaria endemic areas where malaria microscopy or mRDT is unavailable malaria may be over-

diagnosed in febrile patients especially children less than five years of age (Quintana et al., 1998). Diagnosis of

uncomplicated malaria as prescribed by national guidelines is mostly not feasible due to limited infrastructure for microscopy and trained staff. The World Health Organization (WHO) recommends antimalarials such as Artesunate-Amodiaquine (AQ), Artemether-Lumefantrine (AL) or ArtesunateSulphadoxine-Pyremethamine (AS) as first line treatment for uncomplicated malaria in African countries (Kabanywanyi et al., 2007; Nankabirwa et al., 2009) but also proposes the proper diagnosis of the disease for the age groups for rational use and adequate deployment of ACTs. To rationalize the use of expensive ACTs, World Health Organization promotes laboratory confirmation of diagnosis before treatment, for young children in high malaria risk areas where laboratory confirmation is not available. World Health Organization suggests presumptive treatment could be used pending further evidence; the clinical consequences associated with not treating potentially false negative test may outweigh the potential cost-saving benefits.

To enable health facilities and providers to confirm and treat malaria without laboratory, innovative tools such as Rapid Diagnostic Test in the form of a dipstick/cassette using parasite antigen test (Nankabirwa et al., 2009; Wongsrichanalai, Sutamihardja & Wernsdorfer, 2007) are being implemented. However RDTs performance is based on its sensitivity (i.e. its ability for the dipstick to accurately identify true positives) and specificity (i.e. the ability for the dipstick to accurately identify true negatives) which is usually reported by manufacturers. It is thus important to assess the performance of a specific RDT in a different geographical population where malaria parasites may be diverse and also to determine the performance of an RDT at the end user level after procurement and storage. Malaria endemic zones may have a population

characteristic that belies its true sensitivity and specificity in another population. The mRDT can be an important tool to epidemiologists in their quest for global elimination of the malaria disease but epidemiologists must carefully obtain its performance in relation to the community context. MRDTs can help in identifying the parasitaemia in individuals who harbor the *Plasmodium falciparum* even though they are yet asymptomatic (Secardin & Le Bras, 1999)**.** This study intends to evaluate the validity of an RDT that is used in the health facility setting of Kintampo Municipality considering its sensitivity and specificity.

1.2 STATEMENT OF THE PROBLEM

It has been noted that commercially available mRDT kits have shown low sensitivities in products that have performed well on previous occasions (Mtove et al., 2011; Nkrumah et al., 2011). Factors that might affect the validity of a mRDT may differ including species of *Plasmodium* that can be identified. Generally exposure to higher temperature probably seems the major factor for poor performance. High humidity is the second important factor which can degrade mRDT kits rapidly. These factors are present in the Kintampo Municipality which falls within the forest-savanna transitional zone. Out of the three types of mRDT kits, the histidine rich protein (HRP) 2 is the most stable as compared to Plasmodium lactate dehydrogenase (pLDH) and Aldolase. Temperature control, storage, shelf life are therefore important consideration for using mRDT kits in remote areas without electricity by health workers. MRDT kits stored at higher temperatures (especially above 40˚C) are likely to lose sensitivity prior to expiry date (D'Lima & Suslow, 2009). Therefore, it is recommended by the World Health Organization that the sensitivities of approved RDT kits coming into new environments may be

monitored at regular intervals. The sensitivity and specificity of the RDT can be altered significantly depending on the temperature of the area or by the population in which it is tested.

In this study area where temperatures can be as high as 40˚C (Owusu-Agyei et al., 2009), no test has been conducted to determine the sensitivity and specificity of the mRDTs used after procurement of the mRDTs.

In addition, in the study area, malaria transmission is high; a child in the study area has a risk of having about seven (7) malaria infections per year (Owusu-Agyei et al., 2009) and it is likely that a child could test positive after each treatment to mRDT if it is less sensitive or less specific. With the supply of mRDTs for use in facilities in the municipality for diagnosing and treating uncomplicated malaria, increasing numbers of annual malaria cases are being reported with yearly malaria cases rising in the Kintampo municipality with an increase of 13,359 in 2002 to 63,902 in 2011. Several factors may account for this increase. In 2003, the government of Ghana implemented a National Health Insurance scheme to improve access to health care, thus there has been a study increase in outpatient numbers (BLANCHET , 2012), majority of whom are malaria cases (GhanaHealthServiceReport, 2011). Secondly, mRDT has been targeted to be deployed to all health facilities by 2015 to address the widespread problem of poor access to diagnostic testing , thus leading to definitive malaria diagnosis that hitherto may have been misclassified as other diseases (WHO, 2012b, 2013).Other factors such as climate and environmental change, increase in drug resistance, poor living conditions and inadequate control strategies have been attributed to increase in malaria cases in other countries (OECD, 2012). It is possible some of the factors enumerated could account for the rise in OPD cases as observed in the Kintampo municipality but this is unclear. This study seeks to determine the sensitivity and specificity of mRDT that was used in the hospital facility to clarify whether the reporting of malaria cases was

been over diagnosed or under diagnosed. It is against this background that this secondary analysis will confirm the accuracy of the mRDT in correctly identifying the *Plasmodium falciparum* parasite in children seeking care at health facilities in the Kintampo Municipality.

1.3 JUSTIFICATION

Uncomplicated malaria may lead to fatal consequences such as severe disease or death as a result of improper diagnosis. Also the introduction of expensive ACTs makes it imperative for proper diagnosis to avoid malaria parasites developing resistance to these drugs. The findings of this study may establish the validity of the mRDT cassette that may provide evidence to explain the increase in malaria cases over time since 2002 in the Kintampo Municipality.

Since previously approved RDT kits can fail under some environmental conditions and epidemiological settings, the continuous monitoring of the sensitivity and specificity may provide real evidence of stability or otherwise.

1.4 PRIMARY OBJECTIVE OF SCREENING FOR PLASMODIUM FALCIPARUM.

To determine the validity of mRDT cassette in detecting *Plasmodium falciparum* malaria in children aged zero (0) months to 10 years in the Kintampo Municipality.

1.5 SECONDARYOBJECTIVES

(1) To evaluate the sensitivity and specificity of mRDT cassette and compare to the standard field microscopy.

(2) To assess the prevalence of Clinical malaria among children aged 0 months to 10 years.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1 Burden of Malaria

Globally malaria contributes 1.1 million to 2.7 million deaths annually, mostly among children less than five years of age (World Malaria Report, 2009). This is due to the fact that children who are less than five years of age are vulnerable to morbidity and mortality from malaria. In the year 2010, malaria contributed to an estimated 660,000 deaths with an uncertainty range of 490,000 to 836,000 mostly among African children. The prevalence of malaria in sub-Saharan Africa continues to vary among countries that are endemic including Ghana. There was a 29-90% decrease in malaria prevalence between 2001-2007 in The Gambia (Bassat et al., 2008); 80% decrease in Libreville Gabon between 2000-2008 (Guinovart et al., 2008); no consistent trend in Nlakar, Senegal between 1992-2004 (Steketee et al., 2008); 48% increase in Ibadan, Nigeria between 2000-2005 (Chizema-Kawesha E, 2008) and no change in Cote-d'Ivoire between 2002-2005 (Chanda et al., 2009). The declines may be attributed to the use of effective Artemisinin-based Combination Therapies (ACT) control measures as first line treatment for uncomplicated malaria was introduced.

In Ghana the transmission of malaria in the forest region is perennial that is between April and September but peaks in July –August which is a rainy and wet season in most parts of the country including Kintampo, where there is a favorable condition for mosquito vectors and malaria transmission together with the entomological inoculation rate is high, about 270 infective bites per person per year (Dery et al., 2010; Owusu-Agyei et al., 2009) . There has been a general increase (13,359 in 2002 to 63,902 in 2011) in the number of malaria cases reported annually in the district and Children less than five years of age contribute a significant proportion of this number reported.

2.1.2 Prevalence of Clinical Malaria

Malaria transmission and disease burden may vary widely even within a small geographical area (Akogbeto, 1992; Clark, 2008). Every population within an area presents with factors that could decrease or increase the burden of clinical malaria despite malaria control strategies which includes Long-Lasting insecticide-treated bed nets (LLIN) and Intermittent Preventive Treatment in Infants (IPTI).

In other studies risk factors for clinical malaria identified includes renal failure, hepatic involvement, malnutrition and cerebral malaria are strongly associated with complications in severe malaria (Ahmed, Adil, Shahzad, & Yahiya, 2011). Acute renal failure is one of the serious complications of clinical malaria with serious consequences that includes the increased risk of mortality in children. In a study conducted in Uganda (Kiggundu et al., 2013) it was demonstrated that children with severe anemia were at a very high risk of having a malaria parasitemia. But this may not always be the case in certain periods and geographical conditions as observed in Gabon between 2000-2008 where malaria prevalence previously observed was not associated with a significant reduction of anemia burden among children (Bouyou-Akotet et al., 2013).

In Ghana very few studies have been carried out on the prevalence of clinical malaria and these risk factors observed in neighboring African countries are yet to be explored. There are only few studies conducted in communities to date (Donovan, Siadat, & Frimpong, 2012) that explore such risk factors in the middle belt of Ghana. Hence very little is particularly known about the risk factors that may contribute to the prevalence of clinical malaria in Kintampo Municipality. The Ghana Health Service (GhanaHealthServiceReport, 2006) reported that clinical malaria is

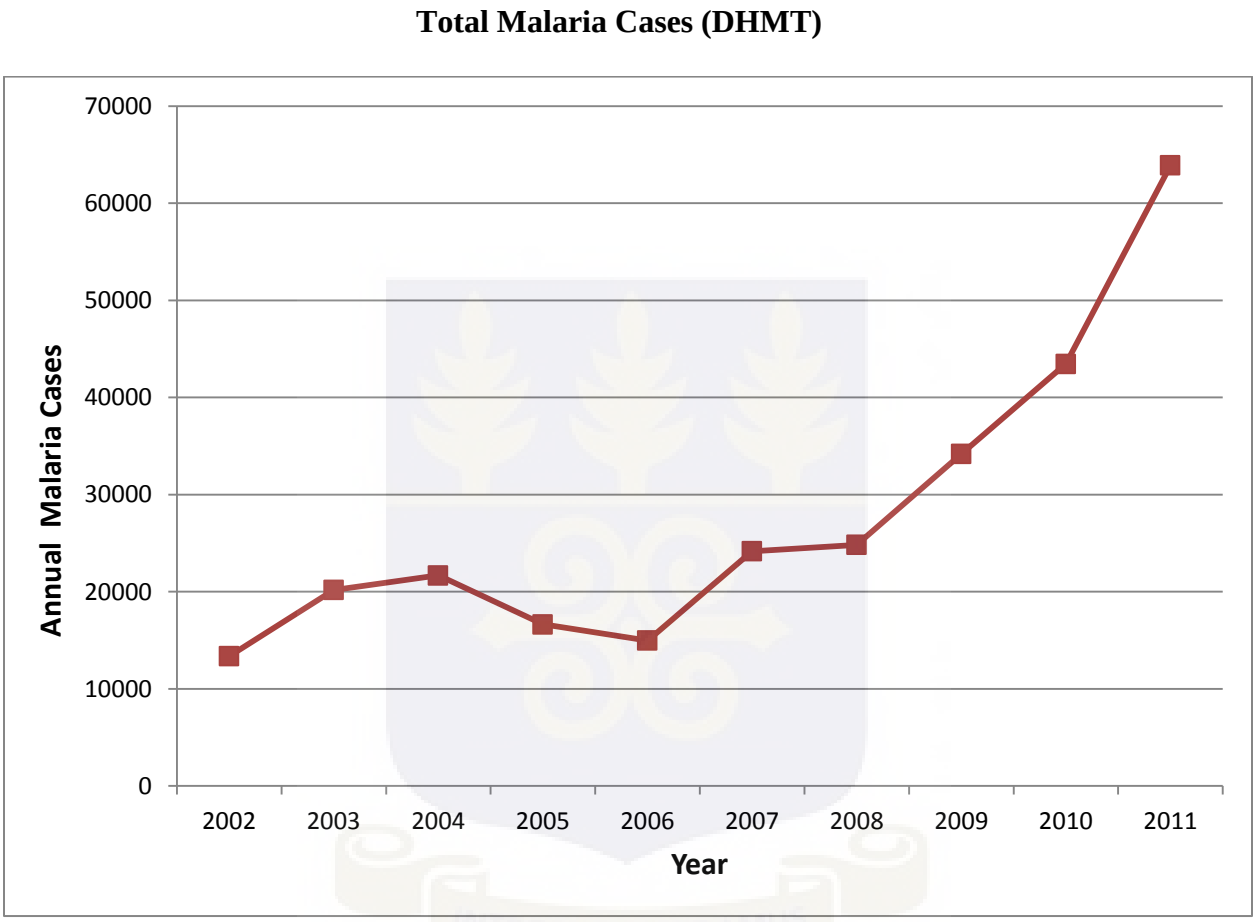
more frequent and severe among children with protein-energy under nutrition and/or micronutrient deficiencies, leading to higher morbidity and mortality due to impaired host immunity. Hence malnutrition has being identified as a significant clinical feature that present in clinical malaria. In this study area of the Kintampo Municipality there is little knowledge on what risk factors are associated with clinical malaria that was observed at the health facility and whether there are other unstudied risk factors that may be present in this particular geographical setting of Kintampo.

2.1.3 Trend of Malaria cases in Ghana

The National Malaria Control Programme (NMCP) reports indicated that there were about 11.3 million cases of Outpatients Department (OPD) malaria in the year 2013 (NMCP, 2013). The translation of this report shows that on average 30,300 of malaria cases were seen each day in the country's health facilities. The proportion of the total OPD cases attributed to malaria gradually reduced from over 45 per cent in the past to less than 40 per cent.

A quick retrospect into the year 2006 when the KMDT was carried out indicated that there was about 30,448,596 reported cases of adult malaria and 7,286,356 reported cases of child malaria in the whole country although it is thought that there were many more cases that went unreported and untreated (MOH, 2011). The direct burden of malaria cases on the sector in terms of out-patient malaria cases remains fairly constant as a proportion of overall cases. However in absolute terms an upward trend has been witnessed since the year 2000, and the number of out-patient cases has risen by around 1,000,000. A trend analysis from the year 2000-2006 shows that an increase in the number of OPD cases of about 2,600,000 in the year 2000; about 3,100,000 in the year 2001; about 3,200,000 in the year 2002; about 3,600,000 in the year 2003; about 3,400,000 in the year 2004; about 3,000,000 in the year 2005; and about 3,500,000 in the year 2006 (MOH, 2011).

Data obtained from the DHMT of the Kintampo Municipality also shows similar trends from the year 2002-2011 on malaria cases. An increase (13,359 in 2002 to 63,902 in 2011) in the number of malaria cases with significant proportion as children under five years of age (which can be seen in the chart below (figure 1).



***DHMT=District Health Management Team**

Figure1: DHMT reports on malaria cases at OPD at Kintampo Municipal Hospital

2.1.4 Strategies to improve case management of Malaria

International calls for home and community management of malaria and for deployment of ACTs have also coincided with new calls for enhanced accuracy in diagnosing malaria **(**Makler,

Palmer, & Ager, 1998) as the guidelines prescribe. The Global malaria control strategy and the action plan for malaria control as stated by World Health Organization has as one of its basic elements to provide early and prompt treatments to persons infected with malaria and targets to reduce malaria mortality and morbidity by at least 20% in at least 75% of the affected countries (Aregawi et al., 2011; Group, 2006). With the action plans translating into research development, policies for parasitological diagnosis of uncomplicated malaria in different epidemiological settings where risk of malaria is high was recommended and adopted by malaria endemic regions including Ghana. The World Health Organization estimates that in most African countries, fewer than 20% of people with suspected malaria in public health facilities are given a diagnostic test (Albertini et al., 2012) and it seems likely that the proportion in private facilities is even lower because they do not understand the operations of the RDTs (GhanaHealthServiceReport, 2009).

2.2 MALARIA DIAGNOSIS USING MALARIA RAPID DIAGNOSTIC TESTAND ITS IMPLICATION

Low parasitological diagnosis for clinical malaria in these endemic zones of Africa lacking in resources is a major barrier to reliable and timely diagnosis (Premji, Minjas, & Shiff, 1994).The absence of resources including microscopes, trained microscopists and power supply has propelled many health facilities to use presumptive treatment. Malaria Rapid Diagnostic Tests are however considered a parasite based diagnostic method because it relies on antigens produced by the parasite (Lau et al., 2011; Sarr et al., 2011). The use of presumptive treatment was recommended over microscopy and RDT test for children under 5 years of age in areas with high malaria prevalence (Bisoffi, Gobbi, Buonfrate, & Van den Ende, 2012). All patients with fever especially in children under 5 years of age in such high transmission areas are treated with ACTs since infections with *Plasmodium falciparum* malaria can lead to rapid death in young children and even in cases that RDTs are done children in high transmission areas are still treated with ACTs since results of RDT test may sometimes be misleading (WHO, 2012a). Despite the challenges with RDTs, it is recommended that febrile patients including suspected treatment failures, severe cases, and pregnant women (Nankabirwa et al., 2009; Zurovac et al., 2008) should be diagnosed before treatment especially in poor resource areas without electricity and microscopy. Malaria diagnosis with RDTs can provide an alternative to microscopy on the basis of its sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) in areas where resources for microscopy including personnel and infrastructure is unavailable (A. H. Moody & Chiodini, 2002; Rowe et al., 2009).

Malaria RDTs are less prone to examiner bias and more dependable in remote areas without electricity. The mRDT cassette that was used to screen participants may be effective in diagnosis of malaria.

2.2.1 Types of RDT Kits

There are currently three types of RDT kits available on the market. These are the histidine rich protein 2 (HRP 2), which is a water soluble protein produced by *Plasmodium falciparum* and specific to *Plasmodium falciparum.* This was the type of RDT that was used in screening for the participants in the original study. The format for the RDT was a plastic cassette that could not break easily in case it fell to the ground and was easy to handle.

Another type is the Plasmodium lactate dehydrogenase (pLDH), currently used in the products that include *Plasmodium falciparum* specific, pan specific and *Plasmodium vivax* specific pLDH. The third type of RDT is the aldolase (pan-specific). Malaria RDTs come in the following formats; Plastic cassettes, cards, dipsticks or a hybrid of cassette and dipstick (Lien et al., 2000)**.** The differences associated with the three types of RDTs reviewed include the type of parasites that can be identified by the RDT. Below is a summary of the specific type of parasites the RDT can identify during diagnosis.

Table 1: Target antigens for commercially available RDTs

Species Antigen	HRP 2	pLDH	Adolase
Faciparum specific	Yes	Yes	No
Pan-specific(All species)	No	Yes	Yes
Plasmodium vivax specific	No	Yes	No

RDTs that are capable of detecting both *falciparum*-specific and non-*falciparum* (or pan-specific) target antigens are commonly called combination or 'combo' tests. Pan-specific means that the RDT detects all the four types of plasmodia that infect humans.

Another difference amongst the types of RDTs is that althougth pLDH tests detecting *P. falciparum* and *P. vivax* (species-specific) are commercially available; their prices are much higher than versions of the same tests using pan-specific pLDH.

2.2.2 Sensitivity and Specificity of RDTs

As per WHO recommendation, the mRDT kits should have a sensitivity of more than 95% parasite density counts of 100 parasites/µL of blood for Plasmodium falciparum (WHO, 2005). This will ensure that all clinically relevant cases of malaria can be detected using these kits as most endemic areas in Africa have a mean parasite density of 500 parasites/µL. Factors affecting the sensitivities of mRDTs include the following; the rates of blood up to the nitrocellulose stripe, adherence of antibodies (Ab) to the stripe, the ability of the antibodies to bind to antigens and the integrity of antibodies-dye conjugate (Kumar et al., 2012).

All these factors are subject to deterioration in the adverse transport and storage conditions. Also the rate of deterioration and their effect can vary between products.

Likewise the specificity of an RDT should be ≥70% so as to make significant impact on diagnosing patients who harbor the parasitaemia (Shabir, 2006). A recently published study estimated that a diagnostic test with 95% sensitivity and 95% specificity requiring minimal infrastructure would avert more than 100,000 deaths and about 400 million unnecessary treatments (Rafael et al., 2006). Patients with clinical malaria are at high health risk because any detected positive should give rise to other testing's, both sensitivity and NPV. The most valuable clinical role of the RDT is in the rapid diagnosis or the exclusion of Plasmodium falciparum

(Stauffer et al., 2009) malaria, which is particularly useful in outpatient settings. By having a high value in sensitivity and negative predictive values, health practitioners know without any doubt that all the negatives are real negatives. The main challenge that confronts RDTs in Africa is the heat stability that has been a consistent obstacle for current malaria RDTs. Some manufacturers have addressed this problem by improving heat stability of some mRDT kits (Ashton et al., 2010; Lee et al., 2012).

The detection of malaria parasites by light microscopy of Giemsa-stained thick and thin blood films remains the standard laboratory method for the diagnosis of malaria (Ransohoff & Feinstein, 1978; Wanji, Kimbi, Eyong, Tendongfor & Ndamukong, 2008) in many malaria endemic zones and can be used to evaluate the validity of mRDT in remote settings by quantifying the species of malaria parasites. RDTs have the disadvantage of identifying incorrectly the status of malaria after clearance of parasites in bloodstream since the antigen histidine-Rich Protein 2 (HRP2) remains in the bloodstream for several days after effective treatment. Also RDTs cannot quantify levels of parasitaemia and species identified in humans; there are however RDTs which could identify more than one *Plasmodium falciparum* species (Flores, Chang & Barillas, 2011).

Sometimes the results of RDTs may be misleading due to certain reasons which are as follows; a negative test results does not always exclude malaria with the certainty because there may be insufficient parasites/antigens to register a positive result, the RDT may have been damaged, illness may be caused by another species of malaria parasites which the RDT is not designed to detect, or in some localities where there is reported widespread of deletions in the parasite gene which produces the antigen which the RDT bases its mechanism of action. In some parts of South America, Uganda, and Senegal, there have been reported cases of deletion in the pf-hrp 2

gene (Wurtz et al., 2013). In these instances, even high parasite density infections will be misdiagnosed as negative and thus affects the sensitivities of the mRDTs.

Also, a positive result does not always signify malaria because antigens may sometimes be detected even after the infecting parasites have died or due to the persistence of malaria gametocytes which does not cause illness, the presence of parasites does not always signify malaria in individuals with high immunity as there may be other causes of fever and finally, presence of other substances (sickle cell anemia prevalence, anti-nuclear antibody positive, rheumatoid factor positive, rapid plasma regain positive, chagas diseases antibody positive, dengue antibody positive, leismaniasis antibody positive and schistosomiasis antibody) positive in the blood may occasionally produce a false positive result.

2.3 MECHANISM OF RDT ACTION

In general, the blood specimen (2 to 50µL) is either obtained from a finger-prick blood specimen, anti coagulated blood, or plasma, and it is mixed with a buffer solution that contains a hemolyzing compound and a specific antibody that is labeled with a visually detectable marker. In some kits, labeled antibody is pre-deposited during manufacture and only a lysing/washing buffer is added. If the target antigen is present in the blood, a labeled antigen/antibody complex is formed and it migrates up the test strip to be captured by the pre-deposited capture antibodies specific against the antigens and against the labeled antibody (as a procedural control). A washing buffer is then added to remove the hemoglobin and permit visualization of any colored lines formed by the immobilized antigen-antibody complexes (Onile, 2005).

2.4 SUPPLY AND USAGE OF RDT IN GHANA

Ghana after adopting the WHO policy for parasitological diagnosis pursued the 3-T policy (test, treat and track). This has not been completely adhered to since not all suspected cases of malaria were tested with either microscopy or a diagnostic kit. Generally service providers are gradually complying with the need to test before treatment and an improvement in the supply of diagnostic materials. Equipping all health facilities with malaria diagnostic facilities (microscopes or RDTs) and provide effective anti-malarial drugs.

In the year 2013 under review, it was anticipated that sixty percent of all OPD malaria cases would be parasitologically tested with microscopy or a diagnostic kit (NMCP, 2013). This target for the country however fell short of expectation with only forty eight percent achieved. The challenge that was mostly encountered country wide was the unavailability of RDTs and personel that will perform such diagnosis. It is however expected that the test rates will increase in future as the campaign for malaria diagnosis is strengthened.

CHAPTER THREE

3.0 METHODS

This was a secondary analysis of data from a study that used a cross sectional analytical study design.

3.1 Description of data source

Baseline enrolment data from the Kintampo Malaria Drug Trial (KMDT) was used for this secondary analysis. The objective of the KMDT was to determine the efficacy and safety of recommended ACTs in Ghana (Artesunate-Amodiaquine, Artemether-Lumefantrine and Artesunate-Chloproguanil). The study was an open labeled randomized trial among children between 6 months and 10 years of age.

Study of the KMDT: The trial was carried out in Kintampo North and South Municipalities of Ghana which covers an area of 7162km^2. The district has a resident population of 140,000 approximately and located in the forest –savannah transitional zone in Ghana where community members are predominantly subsistent farmers (Dery et al., 2010). *Plasmodium falciparum* malaria predominates in this area. Mean monthly temperature range between 18^oC and 38^oC. Malaria transmission in this forest region of Ghana is perennial but peaks in July–August and the entomological inoculation rate is high, about 270 infective bites per person per year (Dery et al., 2010). The prevalence of malaria parasitaemia is greater than 50% among children up to 10 years of age.

Patient identification in the KMDT: Children who visited the Kintampo Municipal Hospital were approached if they had a fever and consented to be part of the study and were screened by a clinician. Patient demographic characteristics such as age and sex and clinical symptoms of patients such as a history of fever within the past two days were recorded on a pretested questionnaire.

Malaria microscopy and mRDT in the KMDT: Capillary blood sample was collected from patients to diagnose the presence of malaria parasitaemia using both malaria microscopy and mRDT. Malaria microscopy was performed by examining 200 oil immersion fields of thick blood films stained with geisma for malaria parasites before a blood film was considered negative. Two expert microscopes examined independently all study slides after the parasite density per micro-litre. They estimated the parasite density by multiplying the number of parasites per 200 leukocytes by a factor of 40, assuming a white blood cell count of 8,000/all. A third microscopist examined the slides if there was disagreement between the two readings**.**

A mRDT was performed by laboratory assistants. A blood specimen (2 to 50µL) from the child's finger-prick was mixed with a buffer solution that contained a hemolyzing compound and a specific antibody that was labeled with a visually detectable marker of colloidal gold. This caused a break in the red cells releasing more parasite protein on the strip, and then bonded the target protein (antigen). If the target antigen was present in the blood, a labeled antigen complex was formed and it migrates up the test strip to be captured by the pre-deposited capture antibodies specific against the antigens. The labeled antibody acted as a procedural control when a washing buffer was added to remove the hemoglobin and permit visualization of any colored lines formed by the immobilized antigen-antibody complexes. If the test was positive this was

indicated by a visible test band antibody and a control band. The test took approximately 10-15 minutes.

Patient treatment in the KMDT: Patients who qualified to be recruited in the study were randomized to receive Artesunate-Amodiaquine, Artemether-Lumefantrine or Artesunate-Chloproguanil. Patients who did not qualify were treated as per the Ghana national treatment guidelines. Result of the primary analysis has been reported elsewhere (Owusu-Agyei et al., 2008).

DATA MANAGEMENT FOR SECONDARY ANALYSIS FOR THIS DISSERTATION

3.2 Data extraction and validity assessment

Data for this secondary analysis of sensitivity and specificity of mRDTs was extracted from an open label, randomized trial of ACTs which sought to determine the efficacy and safety of recommended ACTs in Ghana for the treatment of uncomplicated malaria and conducted by the Kintampo Health Research Centre. Based on this study, a sample size calculation of 937 children giving a power >80% at 95% confidence level to determine a true sensitivity and specificity of not less than 70% was done.

Some variables extracted from the KMDT were re-coded for the purpose of determining the RDT performance that included age, sex, parasite count, hemoglobin and Clinical malaria whiles a univariate and multivariate analysis was done to identify key risk factors of clinical malaria. The data points of interest for determining the performance of the RDT were objective measurements, consistently measured by Standard Operating Procedures (SOPs) that were used to implement the original study.

3.3 Limitations and methodological errors

The major limitation of the secondary data analysis was encountered during recoding. This is because it was difficult to retain the coded data that had already been used for the KMDT. Some important factors could not be represented numerically such as the undetermined values for parasite density greater than 10000. Another limitation was the difficulty to determine if diagnostic tests (RDT) that read as undetermined was actually a different species of malaria parasite. Hence a methodological error of not been able to account for the actual numbers of different malaria species present in the analysis.

Table 2: Variable definition and Measurement

Variable Name	**Data Variable Measurement**	**Secondary Data Analysis Measurement**
Age	Complete age in months	Categorical
Sex	Male/Female	Categorical
Reported fever	Mothers reporting yes/no	Categorical
Measured fever	Complete temperature reading in degrees Celsius>37.5	Categorical
Parasite Count	Complete Parasite count in µl	Categorical
LFT	Normal/Abnormal	Categorical
RFT	Normal/Abnormal	Categorical
Hb	Complete Hemoglobin reading in g/dL	Continuous
WBC	Complete WBC count in µL	Categorical
G6PD deficiency	Normal/Abnormal	Categorical
*Clinical malaria	Yes/No	Categorical

* outcome variable that was generated.

The data used for analysis of the sensitivity and specificity of mRDTs was cleaned and checked for completeness. A tabulation of each variable in the data was used to identify missing data; missing values that were less than 0.5% of the total observations and these were used in the analysis. The data required for this analysis to determine the performance of the RDT was the primary endpoint of the Kintampo Malaria Drug Trial thus provides assurance of its validity in terms of data quality.

3.4 Sample Size

The power to estimate the true sensitivity and specificity using 937 data records was assessed using STATA 11(STATACORP, TX.). It was assumed that the sensitivity and specificity would each be as high as 90% or more (Endeshaw et al., 2012; Hendriksen et al., 2011). The sample of 937 gives a power >80% at 95% confidence level to determine a true sensitivity and specificity of not less than 70% or a true sensitivity and specificity of more than 93%. The assumption considered and used in this study provides a sample size of 937, alpha=0.05 assumed sensitivity or specificity of 90% with a power of 100% and an alternate sensitivity or specificity of 70%.

Table 3: Assumed sensitivity and specificity for the rapid diagnostic tool in Kintampo Municipality

Assumption scenarios	Sample	Alpha	Assumed sensitivity or specificity (ho)	Alternate sensitivity or specificity	Estimated power
Scenario 1	**937**	**0.05**	**90%**	**70%**	**100%**
Scenario 2	937	0.05	90%	85%	99%
Scenario 3	937	0.05	90%	95%	100%
Scenario 4	937	0.05	90%	99%	100%

*Scenario 1 was used as an assumption for the secondary data analysis

3.5 Data analysis

Socio-demographic characteristics of study participants that include age, sex, reported or measured fever, parasite density, liver function test, renal function test, WBC count, hemoglobin and G6PD deficiency were categorical in nature. This was summarized as proportions, while quantitative variables such as hemoglobin was summarized as means based on their distributions.

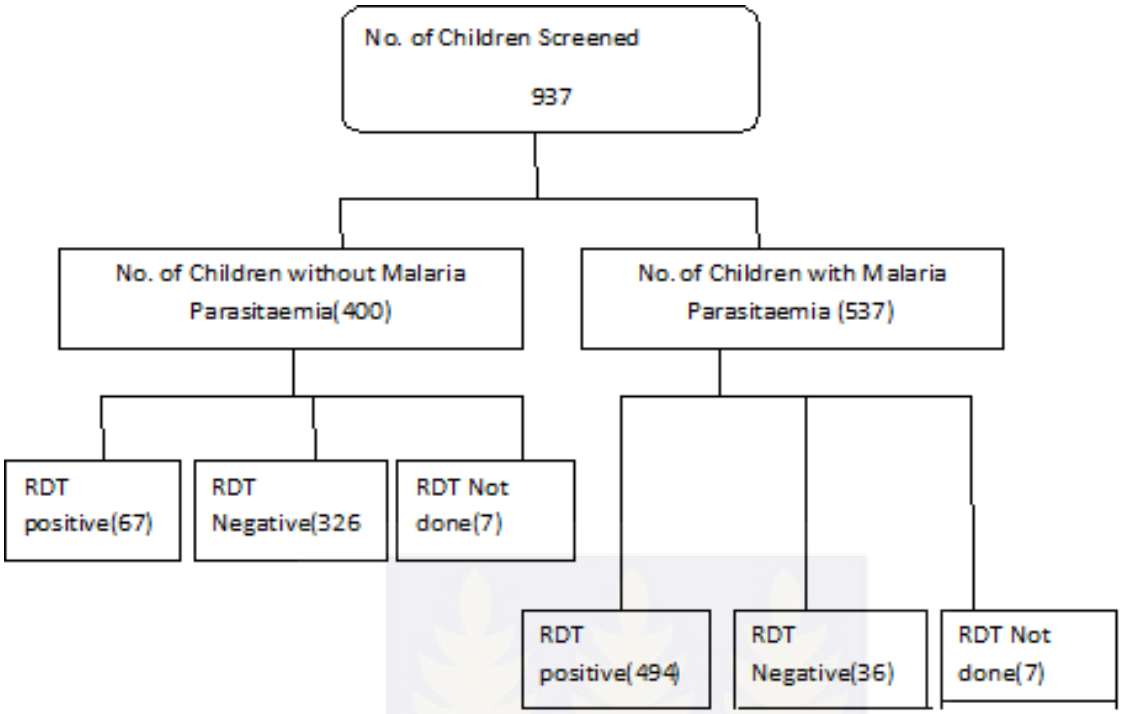
Bivariate analysis was carried out by cross-tabulating baseline characteristics of children with other variables such as RDT and microscopy done together with clinical malaria which is an outcome variable (Table5). Sensitivity and specificity analysis of the RDT used in diagnosing malaria parasitaemia was explored using microscopy as the gold standard. Receiver Operating Characteristic (ROC) was used to determine the ability of the RDT test to correctly classify

patients with or without clinical malaria. The outcome of RDTs was considered as being positive, negative or not done. Specific variables that were measured included true positives (TP), false Positive (FP), true Negative (TN) and false negative (FN). The sensitivity measures the proportion of true positives among all malaria cases using microscopy as a standard for the mRDT. The specificity also measures the proportion of true negatives among all non malarial cases using microscopy as a standard for the mRDT. The probability that a patient has clinical malaria given that the results are positive by mRDT is referred to as its Positive Predictive Value whiles the probability that a patient has no clinical malaria given that the test result of the mRDT is negative is its Negative Predictive Value.

The secondary outcome of this study (clinical malaria) was defined as reported or measured fever with malaria parasite counts >0). Univariate logistic regression was used to explore crude relationships between socio-demographic and potential confounding variables such as age with clinical malaria. All statistical significant variables identified from the univariate analysis were adjusted for in a multivariate logistic regression model. A calculation of 95% confidence interval for the estimates of sensitivity and specificity of the mRDT for malaria variable was done. Also confidence interval for clinical malaria was calculated and P-values (<0.05) were used as a criteria for determining statistically significant associations between clinical malaria and potential risk factors including abnormal liver and renal functions.

For binary exposure variables, the Wald Test P-values were used to determine statistically significant relationship between clinical malaria and potential exposures whiles the likelihood ratio test (LRT) was used to determine statistically significant relationship for exposure variables with more than two levels. The mean and median range for Hemoglobin among children who had clinical malaria and those without clinical malaria was demonstrated as box-plot.

Figure 2: Flow Chart of Participants enrolled for study and by malaria parasitaemia status



No. = Number; RDT= Rapid Diagnostic Test

3.6 Ethical Consideration

The KMDT study protocol was approved by the Ethics Committees of the Kintampo Health Research Centre, Ghana Health Service and the London School of Hygiene & Tropical Medicine. The study is registered at the United States National Institute of Health clinical trials register; with a registration number of NCT00119145.

The research proposal for this secondary analysis was approved by Ghana Health Service Ethical Review Committee.

During the proposal development for this secondary analysis, permission was obtained from the Principal Investigators of the KMDT and from the KHRC to use the data. The data was provided without any identifiers to study children of the KMDT, thus ensuring patient confidentiality. The data obtained from the KMDT investigators is kept confidential by ensuring that all data was password protected from unauthorized users.

CHAPTER FOUR

4.0 RESULTS

4.1 Background Characteristics of Participants

A total of 937 participants screened were used for this secondary analysis. About 99% (923/937) of participants enrolled had both malaria parasitaemia and mRDT done. The children were between ages 0 to 10 years (Mean=2.1years). Over fifty percent (475/934) of them were males.

Most of Children 67.1% (619/923) diagnosed for malaria by both RDT and Microscopy (Table 4) were aged between 12 -59 months). A high proportion 86.3% (797/923) of children reported with WBC count less than 15,000 cells/$mm^3$for children with RDT and microscopy done. Seventy-seven percent (714/923) of children were anemic (hb<10.0 g/dL) (Table 4).

Table 4: Demographic and clinical characteristics of study Participants

Characteristics	No of children with RDT and microscopy done N=923	No of children with no RDT done N=14	Total no. of children screened N=937
Age (months)	n(%)	n (%)	n (%)
0-11	151(16.4)	3(21.4)	154(16.4)
12-59	619(67.1)	7(50.0)	626(66.8)
59+	153(16.5)	4(28.6)	157(16.8)
Sex			
Male	469(50.8)	7(50.0)	416(44.4)
Female	454(49.2)	7(50.0)	461(49.2)
Reported fever			
Yes	917(99.3)	13(92.9)	930(99.3)
No	5(0.5)	0(0.0)	5(0.5)
Not Done	1(0.1)	1(7.1)	8(0.9)
Measured fever			
Yes	541(58.6)	9(64.3)	387(41.3)
No	382(41.4)	5(35.7)	550(58.7)
Measured or Reported Fever			
Yes	918(99.5)	14(100.0)	932(99.5)
No	5(0.5)	0(0.0)	5(0.5)
Parasite Density Parasites/µL			
<5000	159(17.2)	2(14.3)	161(17.2)
5000-10000	58(6.3)	0(0.0)	58(6.2)
>10000	313(33.9)	5(35.7)	318(33.9)
No Parasites	393(42.6)	7(50.0)	400(42.7)
Liver Function Test			
Normal	635(68.8)	7(50.0)	642(68.5)
Abnormal	214(23.2)	3(21.4)	217(23.2)
Not Done	74(8.0)	4(28.6)	78(8.3)
Renal Function Test			
Normal	754(81.7)	10(71.4)	764(81.5)
Abnormal	87(9.4)	0(0.0)	87(9.3)
Not Done	82(8.9)	4(28.6)	86(9.2)
WBC count/µL			
<15	797(86.3)	9(64.3)	806(86.0)
≥15	126(13.7)	5(35.7)	131(14.0)
Hemoglobin (g/dL)			
≤7	169(18.3)	2(14.3)	171(18.2)
7 – 10	545(59.0)	7(50.0)	552(58.9)
>10	208(22.5)	2(14.3)	210(22.4)
Not done	1(0.1)	3(21.4)	4(0.4)
G6PD deficiency			
Normal	380(41.2)	6(42.9)	386(41.2)
Abnormal	82(8.9)	0(0.0)	82(8.8)
Not Done	461(49.9)	8(57.1)	469(50.1)

Note: Participants with characteristics 'Not done' indicates they were excluded from the particular test.

Mean hemoglobin among patients with clinical malaria aged between 0-11 months was marginally high (8.4 g/dL) than those without clinical malaria (7.4 g/dL).Children aged 59 months and above with clinical malaria also reported high mean hemoglobin (10.9 g/dL) than those without clinical malaria (9.9 g/dL) (Figure 3).

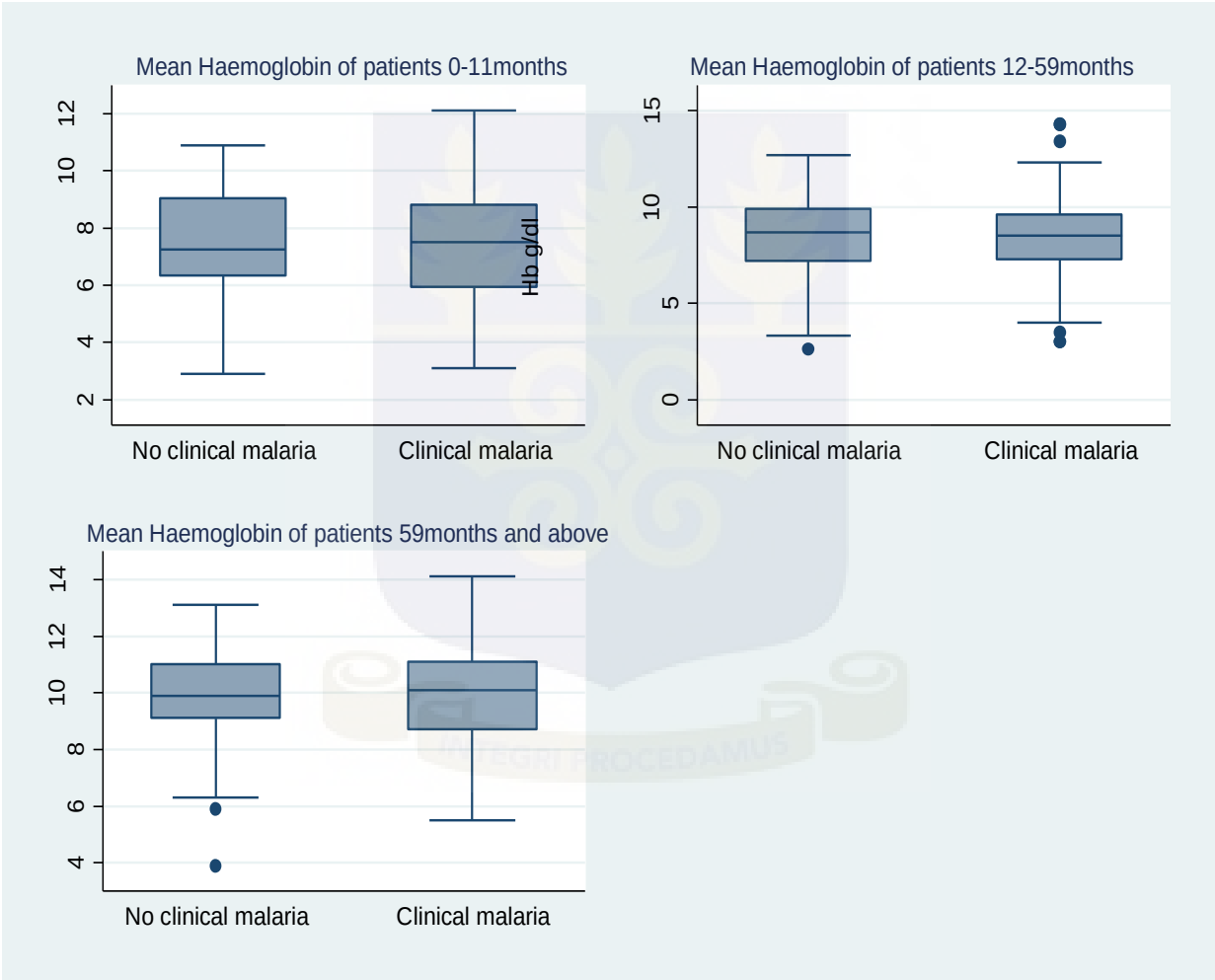


Figure 3: Mean hemoglobin (Hb) among participants with or without clinical malaria in all age groups recruited into the study.

4.2 Sensitivity and Specificity of Malaria Diagnosis by Microscopy and RDT

Overall, using microscopy as the gold standard, the sensitivity and specificity of the RDT used were 93.2% (95% CI: 90.7,-95.2) and 83.0% (95% CI: 78.9-86.5) with a positive predictive value (PPV) of 88.1% (95% CI: 85.1, 90.6) and negative predictive value (NPV) of 90.1% (95% CI: 86.5-92.9) respectively. The mRDT showed both higher sensitivity 94.6% (95% CI: 87.8-98.2) and specificity 88.3% (95% CI: 77.4-95.2) among children aged 0-11 months than among older age groups but not significant since the confidence intervals overlap (Table 5). Sensitivity and specificity of the RDT among anemic children were 96.0% (95% CI: 91.0-98.7) and 60.5% (95% CI: 44.4-75.0%) than among those non-anemic 92.3% (95 % CI: 88.8-94.9) and82.8% (95% CI: 77.4-87.4) respectively. The sensitivity and specificity were generally higher among patients with abnormal liver function test (LFT) and with those with abnormal renal function test (Table 5).

Table 5a: Sensitivity, specificity, and predictive values of mRDT using malaria microscopy as a gold standard

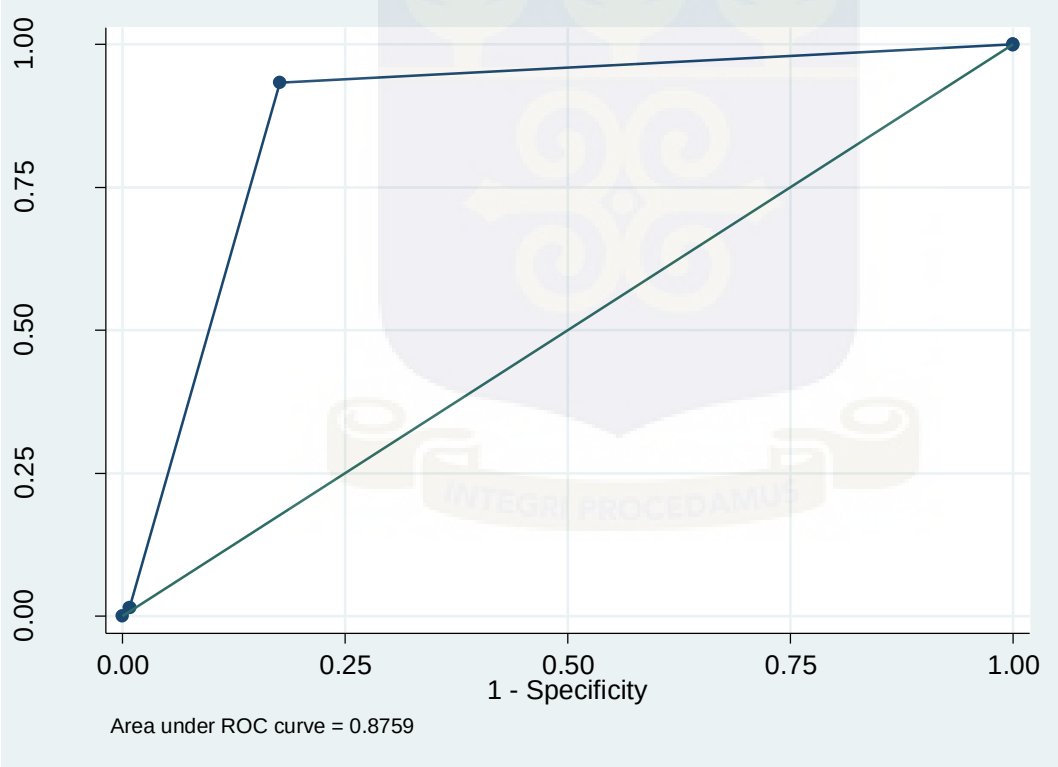
Characteristics	Microscopy Positive		Microscopy Negative					
	mRDT + (n)	mRDT -(n)	mRDT + (n)	mRDT -(n)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Overall	326	67	36	494	93.2(90.7-95.2)	83.0(78.9-86.5)	88.1(85.1-90.6)	90.1(86.5-92.9)
Age (months)								
0-11	87	5	7	53	94.6(87.8-98.2)	88.3(77.4-95.2)	92.6(85.3-97.0)	91.4(81.0-97.1)
12-59	346	27	46	208	92.8(89.6-95.2)	81.9(76.6-86.4)	88.3(84.7-91.3)	88.5(83.7-92.3)
59+	73	6	14	66	92.4(84.2-97.2)	82.5(72.4-90.1)	83.9(74.5-90.9)	91.7(82.7-96.9)
Sex								
Male	255	23	39	155	91.7(87.8-94.7)	79.9(73.6-85.3)	86.7(82.3-90.4)	87.1(81.2-91.6)
Female	251	15	28	172	94.4(90.0-96.8)	86.0(80.4-90.5)	90.0(85.8-93.2)	92.0(87.1-95.4)
Reported Fever								
Yes	322	67	38	505	93.0(90.5-95.0)	82.8(78.6-86.4)	88.3(85.4-90.8)	89.4(85.8-92.4)
No	4	0	0	1	100(2.5-100.0)	100.0(39.8-100.0)	100.0(2.5-100.0)	100(39.8-100.0)
Measured Fever								
Yes	324	25	32	167	92.8(89.6-95.3)	83.9(78.1-88.7)	91.0(87.5-93.8)	87.0(81.4-91.4)
No	182	13	35	160	93.3(88.9-96.4)	82.1(75.9-87.2)	83.9(73.3-88.5)	92.5(87.5-95.9)
Parasite Density/µL								
<5000	125	29	2	3	81.2(74.1-87.0)	60.0(14.7-94.7)	98.4(94.4-99.8)	9.4(2.0-25.0)
5000-10000	55	2	1	0	96.5(87.9-99.6)	0.0(0.0-97.5)	98.2(90.4-100.0)	0.0(0.0-84.2)
>10000	-	-	-	-	-	-	-	-
WBC Count /µL								
<15	287	26	36	202	91.7(88.1-94.5)	84.9(79.7-89.2)	88.9(84.9-92.1)	88.6(83.7-92.4)
>15	217	12	31	124	94.8(91.0-97.3)	80.0(72.8-86.0)	87.5(82.7-91.3)	91.2(85.1-95.4)

*Parasite Density >10000 was missing in all the cell; PPV=Positive Predictive Value; NPV=Negative Predictive Value; WBC=White Blood Cell ; CI= Confidence Interval

Table 5b: Sensitivity, specificity, and predictive values of mRDT using malaria microscopy as a gold standard

Characteristics	**Microscopy Positive**		**Microscopy Negative**					
	mRDT + (n)	mRDT -(n)	mRDT + (n)	mRDT -(n)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Hemoglobin (g/dL)								
≤7	121	5	17	26	96.0(91.0-98.7)	60.5(44.4-75.0)	87.7(81.0-92.7)	83.9(66.3-94.5)
7 – 10	299	25	40	193	92.3(88.8-94.9)	82.8(77.4-87.4)	88.2(84.3-91.4)	88.5(83.5-92.4)
>10	85	8	10	108	91.4(83.8-96.2)	91.5(85.0-95.9)	89.5(81.5-94.8)	93.1(86.9-97.0)
G6PD deficiency								
Normal	21	5	19	335	80.8(60.6-93.4)	94.6(91.796.7)	52.5(36.1-68.5)	98.5(96.6-99.5)
Abnormal	4	1	7	70	80.0(28.4-99.5)	90.9(82.2-96.3)	36.4(10.9-69.2)	98.6(92.4-100.0)
Liver Function Test								
Normal	361	29	39	220	92.6(89.5-95.0)	84.9(80.0-89.1)	90.3(86.9-93.0)	88.4(83.7-92.1)
Abnormal	125	3	21	66	97.7(93.3-99.5)	75.9(65.5-84.4)	85.6(78.9-90.9)	95.7(87.8-99.1)
Renal Function Test								
Normal	454	32	49	233	93.4(90.8-95.5)	82.6(77.7-86.9)	90.3(87.3-92.7)	87.9(83.4-91.6)
Abnormal	28	1	7	51	96.6(82.2-99.9)	87.9(76.7-95.0)	80.0(63.1-91.6)	98.1(89.7-100.0)

The Receiver Operating Characteristics (ROC) classifies subjects into one of two categories usually diseased or non-diseased. In this case children were classified as those with malaria parasitaemia and those who did not. The true disease status is often referred to as the gold standard (microscopy). As accuracy improves, the Area Under Curve (AUC) approaches one. AUC is an overall accuracy of the mRDT kit which is interpreted as; an AUC=0.5 indicates that ROC corresponds to random chance while an AUC=1 corresponds to perfect accuracy. Hence the AUC= 0.88 indicates that the mRDT test has 88.0% accuracy of classifying children who had malaria parasitaemia and those who did not and this was a good indication of the mRDT (figure 4).



*ROC= Receiver Operating Characteristic

Figure 4: Receiver Operating Characteristic (ROC) for malaria parasitaemia using RDT tool among 937 patients.

The prevalence of clinical malaria in the children recruited in the KMDT was 56% (550/973). The prevalence of clinical malaria was 61.0%, 59.0% and 55.4% among children in 0-11 months, 12 – 59 months and >59 month age groups respectively (Table 6).

Sex and age were considered as important variables related to clinical malaria apriori. The risk of clinical malaria was 0.81 times higher among children aged 12-59 months patients than those who were 0-11 months old patients but not significant [OR 0.81 (95% CI: 0.55-1.21), p=0.30]. Also the risk of clinical malaria was 0.89 times higher in female children than children who were male [aOR 0.89 (95% CI: 0.67-1.17), p=0.40] . The risk of clinical malaria was 1.45 times higher among children with abnormal liver function test than among children with normal liver function test [aOR 1.45 (95% CI:1.05-2.01), p=0.03] after adjusting for age and sex in the Multivariate logistic regression model. There was also a strong association between renal function test and clinical malaria: children with abnormal renal function test were less likely to experience clinical malaria [aOR=0.55 (95% CI: 0.34-0.87, p=0.01) respectively (Table 6).

Table 6: Univariate and Multivariate logistic regression of predictors of clinical malaria among children

Characteristics	Clinical malaria	No clinical malaria	Total no. of children screened	Univariate OR 95% CI	P*	Multivariate aOR 95% CI	P*
Age (months)	n(%)	n (%)	n (%)	-	-	-	-
0-11	94(61.0)	60(39.0)	154(100.0)	1		1	-
12-59	369(59.0)	257(41.1)	626(100.0)	0.92(0.64-1.31)	0.64	0.81(0.55-1.21)	0.30
59+	87(55.4)	70(44.6)	157(100.0)	0.80(0.51-1.25)	0.32	0.68(0.41-1.10)	0.11
Sex	-	-	-	-	-	-	-
Male	288(60.5)	188(39.5)	476(100.0)	1	-	1	-
Female	262(56.8)	199(43.2)	461(100.0)	0.86(0.66-1.11)	0.25	0.89(0.67-1.17)	0.40
WBC Count/µL	-	-	-	-	-		-
<15	470(58.3)	336(41.7)	806(100.0)	1	-	-	-
≥15	80(61.1)	51(38.9)	550(100.0)	1.11(0.76-1.63)	0.59	-	-
Hemoglobin g/Dl	-	-	-	-	-	-	-
≤7	98(57.3)	73(42.7)	171(100.0)	1	-	-	-
7 – 10	333(60.0)	225(40.0)	563(100.0)	1.12(0.79-1.58)	0.53	-	-
>10	116(54.5)	97(45.5)	213(100.0)	0.89(0.59-1.33)	0.58	-	-
G6PD deficiency	-	-	-	-	-	-	-
Normal	259(64.6)	142(35.4)	401(100.0)	1	-	-	-
Abnormal	50(61.0)	32(39.0)	82(100.0)	0.86(0.53-1.40)	0.54	-	-
Liver Function Test	-	-	-	-	-	-	-
Normal	371(56.6)	285(43.5)	656(100.0)	1	-	-	-
Abnormal	141(64.7)	77(35.3)	218(100.0)	1.41(1.02-1.93)	0.04	1.45(1.05-2.01)	0.03
Renal Function Test	-	-	-	-	-	-	-
Normal	462(59.4)	316(40.6)	778(100.0)	1	-	-	-
Abnormal	41(47.1)	46(52.9)	87(100.0)	0.61(0.39-0.95)	0.03	0.55(0.34-0.87)	0.01

OR=Odds Ratio ; P*=P-value ; CI=Confidence Interval ; WBC= White Blood Cells
aOR=adjusted Odds Ratio

CHAPTER FIVE

5.0 Discussion

There was generally a good indication of sensitivity and specificity of mRDT kit in the study population as the tests discriminated those with malaria parasitaemia against those who do not. However the finding from this study is slightly similar to what was observed in Tanzania (93.0% vs. 90.0% and 96.6% vs. 83.0%) (Mboera et al., 2006)**.** The reason for the slight differences in sensitivity, specificity, PPV and NPV may be attributed to the higher prevalence of malaria in the Kintampo districts and other possible genome factors present in the population characteristics such as anemia and G6PD deficiency status. There is slightly lower sensitivity difference of 2.0% recorded by the mRDTin this study than the World Health Organization criteria of sensitivity, which is recommended should be above 95% when compared to microscopy (A. Moody, 2002).This can be explained that at low levels of parasitaemia in patients, the sensitivity of the RDT may fall below expected requirements. This is of concern since non-immune individuals without symptomatic malaria can occur at parasite densities that are below the detection threshold of currently available RDTs. Sometimes storage conditions of mRDT kits could also affect their sensitivity. Also misclassification of readings is a possible factor for low sensitivity as quality control measures were put in place such as double reading and confirmation of a third reader to minimize such errors. However, the level of specificity was acceptable in accordance with WHO standard and also slightly higher in studies that have been conducted in the same study area but with a different brand of the mRDT (Baiden et al., 2012). The sensitivity of the mRDT is higher when malaria parasites counts higher than 5000/micro liter of blood. This is consistent with reports by the WHO which suggests that the sequestration increases the level of available antigen concentration that binds with the mRDT. It is likely that the mRDT truly

identify malaria parasiteamia when the parasites available in the blood stream are high. The main test properties of diagnosing clinical malaria depend on the prevalence that is measured.

The implications for the wrong classification may lead to Clinicians giving wrong treatments to patients who present with symptoms of other diseases related to clinical malaria and must carefully be interpreted. This could increase the resistance to ACTs that are expensive in a vulnerable population of children. The specificity I report in this study indicates that mRDT could miss patients that have malaria parasitaemia with false negative indication. Low parasite densities are a factor for wrongly classifying patients as not having malaria but the study is not adequately powered to make this clear (McMorrow, Aidoo, & Kachur, 2011)

5.1 Clinical Malaria in Children Population

The prevalence of clinical malaria among the children recruited is high in all the age groups but higher in the younger age group (0 -11months). It is likely that the younger age group have higher parasite loads since they have the least immunity to malaria. This pattern of prevalence in the age groups in this study is similarly reported in a study by Snow and Marsh in 2002 where the higher prevalence of clinical malaria in the 0-11 month's ages reflects increasingly rapid acquisition of immune responses that limit the life-threatening effects of malaria with increasing exposure to the parasite.

In this study there is also a high risk of malaria infection amongst abnormal renal infection patients. This is unexpected since cases recruited are uncomplicated malaria with *Plasmodium falciparum* infection. *Plasmodium malariae* infection is known to cause chronic renal

impairment while *Plasmodium falciparum* causes acute renal impairment in complicated malaria. In the study area, majority (98%) of infections is *Plasmodium falciparum (*Dery et al., 2010). It is

likely that, the patients with abnormal renal function test have a mixture of *Plasmodium falciparum* and *Plasmodium malariae* infections. In other Asian studies conducted, factors found that explain why the risk of clinical malaria was associated with renal infections included fever clearance time and anemia (Tangpukdee, Elshiekh, Phumratanaprapin, Krudsood & Wilairatana, 2011; Vannaphan et al., 2010). The complications of clinical malaria of the factors mentioned could lead to fatal outcomes including mortality in a vulnerable population of children.

5.2 Limitations

Aside the usual limitations associated with secondary data analysis which includes lack of influence on the study design and methods used for data collection, the data answered the research objective of this study; determining the performance of the RDT used to screen children for the drug trial. However the study design limitations restricted the study's ability to determine the effect of genetic factors (individual and parasite population) and gametocyte carriage on the sensitivity of the RDT kit. Also, usual requirement of PCR testing for other malaria and other pathology in the cases of parasite negative samples was not undertaken. Also, HRP 2 sequencing

by PCR amplification to determine if parasites could produce the detecting antigen was not carried out hence a situation where there could be an underestimation or over estimation of both sensitivity and specificity. Reproducibility of the results of the mRDT was not performed as the same or different reader may not achieve the same results on repeated samples. Reproducibility testing on the mRDT should have been considered and conducted in a blinded fashion that is

readers should not know the results obtained previously. However the original study did not use mRDT as the main determinant of malaria parasites. This measure could have provided important evidence to the closeness of agreement between test results when the conditions for testing or measurement change. Inter-observer variability of the mRDT may have increased if the study were set out to measure such specific objectives in the analysis

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

This mRDT maintains its high sensitivity and specificity among patients in the forest-savanna transitional zone of Ghana. The prevalence of clinical malaria among children who were screened for the KMDT was generally high, but highest among younger children (0-11months).

RDTs as a diagnostic instrument for malaria in Outpatient department in Kintampo Municipality is a good tool that will augment the screening of patients to decrease the prevalence of clinical malaria by targeting those who need ACTs the most and prevent resistance.

6.2 RECOMMENDATIONS

The result of the study has shown that the sensitivity and specificity of the mRDTs is high. Clinical malaria among children less than 10 years who presented with cases at the clinic was prevalent in the study area, hence the following recommendations drawn per the outcome of the study.

- It may be important that malaria cases reported in facilities be supported by sound evidence of diagnosis before taking further decisions by clinicians. The DHMT should strengthen policies regarding diagnosis of malaria in the Kintampo Municipality especially in children aged 0month to ten years.

- The cassette should be provided to peripheral public health facilities by the Ministry of Health without trained laboratory technicians, electricity, and well equipped laboratory to correctly identify, monitor and complement parasitological diagnosis where there is high prevalence of uncomplicated malaria in the population.

- Further studies will need to be conducted to access the relationship between clinical and abnormal renal function. This is because antimalarial drugs that are contraindicated in patients with renal impairement will have to be administered with care. A confirmation of these results requires a renal function test of all malaria patients if a drug with poor excretion profile is to be administered.

APPENDICES

CURRICULUM VITAE

PERSONAL INFORMATION

Surname: Boateng

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Date of Birth: November 17, 1986

Nationality: Ghanaian

Gender: Male

Languages Spoken**:** English, Twi, Ga

CONTACT INFORMATION

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ACADEMIC BACKGROUND/EDUCATION

Bachelor of Science in Computer Science and Statistics, University Of Ghana- Legon; 2009

Senior high School Certificate, Labone Senior Secondary School; 2004

POSTGRADUATE WORK: MAJOR/MINORS

Data Management and Analysis/Reporting and Reviewing Clinical Trials, Special Electives in EPDC, Clinical Trials in Practice, Principles of Epidemiology, Protocol Development, Trial Designs, Regulatory Issues, Good Clinical and Laboratory Practice, Project Management and Resource Coordination , Advance Statistical Methods in Clinical Trials, Cluster Randomized Trials, Design and Analysis of Epidemiological Studies

PUBLICATIONS (LOCAL)

INDEPTH (Phase IV) Effectiveness and Safety Studies of Antimalarials in Africa.

Feasibility of Biometric identification Techniques for Demographic Surveillance System in an African Rural setting

PRESENTATION(S)

INDEPTH (Phase IV) conference for data managers (De res Salem, Tanzania)

WORK EXPERIENCE

Research Officer: Kintampo Health Research Centre, Ghana Health Service, Ghana- August 2009

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Appendix 2:

KINTAMPO HEALTH RESEARCH CENTRE GMP 2,MALARIA DRUG TRIAL, APRIL 2005 SCREENING AND ENROLMENT FORM	FORM NO.	FORMNO

PART A: IDENTIFICATION DATA

1. Screening Number…… | | | | | SCNO

2. Name of child _____ CNAME

3. Initials of the study child (first two letters of the first and last names)……. | | | | | INITI

4. Child's Date of Birth (dd/mm/yy) …… | | | | | | | DOB

4a. Age…….... | | YEARS + | | MONTHS

4b. Source of age

CHILD CARD	1	PERMANENT KDSS ID CARD	2	REPORTED WITHOUT PROMPTING	3	SAGE
PROMPTED WITH EVENTS CALENDER	4	OTHER RATING RECORDS	5			

5. Sex of child…… MALE 1 | FEMALE 2 | SEX

6. Date of exam. (dd/mm/yy) …… | | | | | | | DOE

7. Name of the mother _____ MNAME

8. Compound Number …… | | | | | | | COMPNO

9. Childs permanent KDSS number | | | | | | | | | | | | PERMID

PART B. CLINICAL HISTORY & EXAMINATION

ASK WHETHER THE CHILD HAS ANY OF THE FOLLOWING SYMPTOMS. WRITE 99 FOR DURATION OF SYMPTOMS IF THE ANSWER TO SYMPTOM ENQUIRY IS "2 = NO" OR "8 = DON'T KNOW".

10. Is the child unable to suckle/drink?……	YES	**1**	NO	**2**	DK	**8**	DRINK
10a. If yes, how many days? ……							DDINK
Has the child had**:**							
11. Fever within the past 2 days……..	YES	**1**	NO	**2**	DK	**8**	FEV
11a. If yes how many days? ……							DFEV
12. Runny nose. ……	**YES**	1	**NO**	2	**DK**	8	RNOSE
12a. If yes, how many days?……							DRNOS
13. Cough. ……	YES	1	NO	2	DK	8	COU
13a. If yes, how many days……							DCOU
14. Difficulty in breathing. ……..	YES	1	NO	2	DK	8	DBRE
14a. If yes how many days……							DDRE
15. Diarrhoea. ……..	YES	1	NO	2	DK	8	DIA

Appendix: 3

PART C. BLOOD SAMPLES COLLECTED

48. Blood slide for malaria parasite ……	**YES**	1	**NO**	2		BSLID
49. Filter paper sample for PCR and antimalarial drug assays……..	**YES**	1	**NO**	2		FILT
50. Blood sample for malaria mRDT test……..	**YES**	1	**NO**	2		OPT
51. Blood sample in microtainers for hb, cell count, LFT, RFT, G6PD	**YES**	1	**NO**	2		BSM
52. Malaria mRDT test	POSITIVE	1	**NEGATIVE**	2	**UNDETERMINED** 9	DIP

PART D: ELIGIBILITY CRITERIA

If malaria mRDT test is positive request the lab to do the relevant tests urgently and assess the eligibility of the child for enrolment in the study

53. Blood slide for *Pf* malaria	POSITIVE	1	**NEGATIVE**	2	**UNDETERMINED** 9	BSR
54. Parasite density >2000 and <200000 /µl	**YES**	1	**NO**	2	**UNDETERMINED** 9	DENSE
55. Hb≥ 7 gm/dL ……	**YES**	1	**NO**	2	**UNDETERMINED** 8	HB
56. Total WBC ≤ 15000/µl…	**YES**	1	**NO**	2	**UNDETERMINED** 8	WBC
57. Liver function test ……..	**NORMAL**	1	**ABMORMAL**	2	**UNDETERMINED** 8	LFT
58. Renal function test…….	**NORMAL**	1	**ABMORMAL**	2	**UNDETERMINED** 8	RFT
59. Body weight >5 kgs……	**YES**	1	**NO**	2		WEIGT
60. Age > 6 months to 10 years……	**YES**	1	**NO**	2	**NS** 8	AGE2

61.Temperature ≥37.5°C or history of fever within 24 hours	**YES**	1	**NO**	2	**NS**	8	TEMP2
62. Diagnosis uncomplicated *Pf malaria*……..	**YES**	1	**NO**	2	**NS**	8	DIAG
63. Child has never participated in this trial…….	**YES**	1	**NO**	2	**NS**	8	PART
64: Is the child eligible for enrolment…… .	**YES**	**1**	**NO**	**2**	**NS**	**8**	**ELIG**
65. G6PD Status……	**NORMAL**	1	**DEFICIENT**	2	**NS**	8	G6PD