EVALUATION OF CHILDHOOD MALARIA MANIFESTATIONS, DIAGNOSTIC AND MANAGEMENT PRACTICES IN WESTERN KENYA HIGHLANDS

BY

JOSEPH KIPROP CHOGE

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PARASITOLOGY OF THE UNIVERSITY OF ELDORET

OCTOBER, 2013

DECLARATION

DECLARATION BY THE STUDENT

This thesis is my original work and has not been submitted for the award of any degree in any other university. No part or whole of this research study should be produced without my consent or that of School of Science, University of Eldoret.

Name of Student: **Joseph Kiprop Choge**

Registration Number: **SC/ D.Phil./002/2008**

Signature: …… Date: …….....

DECLARATION BY THE UNIVERSITY SUPERVISORS

The thesis entitled "*Evaluation of Childhood Malaria Manifestations, Diagnostic and Management Practices in Western Kenya Highlands"* is a research thesis that was submitted to the School of Science of the University of Eldoret, with our approval as the University Supervisors.

1. **Prof. F. Esamai.MMed; MPH, PhD.**

   Professor of Child Health and Paediatrics; Principal, School of Medicine, Moi University.

   Signature…….Date……

2. **Dr. A.G.M Ng'wena.PhD.**

   School of Medicine, Moi University

   Signature…….Date……

3. **Dr. M.M Ngeiywa.PhD.**

   School of Science, Moi University

   Signature…….Date……

DEDICATION

To my beloved wife, Lena, sons and daughters: Jennifer Chebet, June Chepchirchir, Bethuel Kibichiy, Japheth Kipkorir, and Sifa Jepchumba, who endured many difficult situations but ensured I had peace and one piece of mind to complete my entire Doctorate work. Only God can reward you all for your help. I specially dedicate the untiring efforts of my mother, Truphena, who sacrificed much for the sake of my education. I also acknowledge my brother, Simon and his entire family for their immense support.

In memory of all who have in one way or other contributed in the past, present or will in future contribute to the efforts to cure, control or eradicate malaria, I dedicate this quote to you, which was cited from *The Prayer of Jabez, Devotional* ; Mulmomah Publishers, Inc. © 2001by Bruce Wilkinson.P.23:

'If you think you are too small to be effective, you have never been in bed with a mosquito.'

- Betty Reese-

ABSTRACT

Malaria remains a major killer in many parts of the world and challenges in accurate malaria diagnosis regrettably encourage widespread presumptive malaria treatment. The study conducted between September 2009 and May 2011 compared the sensitivity of Immunochromatographic malaria rapid diagnostic test with microscopy as the gold standard, using plasma, whole blood and serum among affected children (aged <12 years). Malaria rapid diagnostic test training and user-attitudes among healthcare professionals in the highlands and lowlands of western Kenya and malaria management practices in highland-based health centres were evaluated and determination of predominant malaria features, malaria parasite densities and the correlation of the latter with haemoglobin levels were also done. The results confirmed that immunochromatographic (ICT) malaria rapid test had 97% specificity to *Plasmodium falciparum*, while the 3% that tested negative using ICT were microscopically identified as *Plasmodium malariae* (1.5%) and *Plasmodium ovale (1.5%)*, respectively. No child in the sample population had *Plasmodium vivax*. There was a highly significant difference in the species occurrence of malaria parasites (Fisher's exact test; $p<0.0001$).The sensitivity of ICT when using plasma and whole blood was the same (86.4%) but was comparatively less when serum was used (84.8%). There was a highly significant difference between the training and user-attitudes of health professionals in the highlands and lowlands of western Kenya towards rapid diagnostic tests (Pearson Chi Square; Pr <0.001). Fever, malaria-associated anaemia and sweating were elicited among 92.4%, 85.6% and 81.1% of the affected children, respectively. Pearson's analysis showed a negative correlation between haemoglobin levels and malaria parasite density but the negative correlation was statistically insignificant ('R'= -0.1171; p =0.181). Out of 153,530 patients evaluated for possible malaria across five highland-based health centres during 2001-2010 periods, 58.3% were presumptively treated, 33.3% had no malaria parasites and 8.4% had malaria parasites. Paired sample T- test showed a highly significant difference between the presumptively treated and laboratory-confirmed malaria across three (n=9771; mean=1.132E3; 95% C.I: 918.517 - 1.346E3; $p < 0.001$) and across two (n=118,255; mean=8119.571; 95% C.I: 5836.924-10402.219; $p <0.001$) highland-based health centres in western Kenya during years 2007 and 2001-2007, respectively. Immunochromatographic rapid diagnostic test is significantly sensitive and specific to *Plasmodium falciparum*; hence should be used to support microscopy method of malaria diagnosis. Although whole blood, plasma or serum is recommended for malaria diagnosis using immunochromatographic test, serum is less reliable than plasma or whole blood. Urgent training to sensitize qualified health professionals, especially in the highlands of western Kenya, to effectively use malaria rapid diagnostic tests, is vital. Investigations to establish and manage the causes of the high anaemia prevalence among children (aged twelve and below) with malaria, are urgently required. Measures to improve and update malaria morbidity statistics of children, to reduce presumptive malaria treatment and increase reliance on laboratory diagnosis of malaria should be identified and implemented.

Key Words: **Anaemia; Haemoglobin; Malaria; Parasite density; Plasma; Rapid diagnostic tests; Serum; Whole blood**

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT :	Artemisinin-based Combination Therapy
CDC:	Centers for Disease Control
C.I :	Confidence Interval of the differences
DCDC:	Division of Communicable Disease Control (Ministry of Health, Kenya)
DVBD:	Division of Vector-Borne Disease
EDTA:	Ethylenediaminetetraacetic acid, widely abbreviated as EDTA, is apolyamino carboxylic acid and is used extensively in the analysis of blood. It is an anticoagulant for blood samples for complete blood count (Lanigan and Yamarik, 2002).
HRP-II (HRP-2):	Histidine-Rich Protein II
ICT:	Immunochromatographic Test
IMCI:	Integrated Management of Children Illness
IRS:	Indoor Residual Spray
ITN:	Insecticide Treated Net
KEMRI:	Kenya Medical and Research Institute
MOH:	Ministry of Health
MRDT:	Malaria Rapid diagnostic test
PLDH:	Pan-malaria Lactate dehydrogenase (LDH)
PCR:	Polymerase Chain Reaction
QBC:	Quantitative Buffy Coat
RDT:	Rapid diagnostic test
WHO:	World Health Organization

ACKNOWLEDGEMENT

I wish to acknowledge in a special way, Professor Fabian Esamai, Professor of Paediatrics and Childhealth, who is also serving as the Principal, School of Medicine, Moi University, for his precious mentorship. I especially thank him for not only supervising my masters and doctorate research work but for emphasizing during his inaugural speech on 27th September, 2007 that '*the application of simple and feasible preventive strategies, adoption of evidence based medicine and utilization of the results and findings of essential health research provide the foundation for reaching the desired impact of reducing child mortality'.* That phrase inspired me to immediately register for the doctorate work that culminated in this thesis. I also thank Dr. Gideon N. Magak, School of Medicine, Moi University, for supervision and keenly following on the progress of my field work. I thank Dr.M.M. Ngeiywa, School of Science of Chepkoilel University College for his supervision, mentorship and teaching since the days of my undergraduate studies.

In a very special way I thank Dr. Willis Akhwale, the Director of Disease Prevention and Control, Ministry of Public Health and Sanitation, Kenya, for having inspired me since our high school days at Starehe Boys' Centre and School to date. He personally took great effort to ensure I got malaria rapid diagnostic tests for the current study, from Surgipharm Pharmaceuticals, Kenya. Many thanks to all those who also helped me through other processes during the entire research period, including Mr. Kothari (Surgipharm), the Chief Clinical Officer and Registrar of Clinical Officers' Council, Mr. Kisoo, Mr. Wesley Tomno (National Chairman, Clinical Officers' Association) and Mr. Julius Kimitei (Division of Malaria Control, Kenya) among others. I wish to thank Dr.

Edward Serem, Medical Superintendent, Kapsabet District Hospital for creating an enabling environment for my research within his administrative area of jurisdiction.

In a special way, I also acknowledge my mentor, personal friend and former K.M.T.C collegemate Prof. Charles Obonyo, Professor, Center for Vector Biology & Control Research, KEMRI, Kisumu (Kisian), and Maseno University Professor, for spending much of his precious time to personally encourage me. Our three years spent engaging in weekly Bible studies with Prof. Dankit Nassiuma, the immediate former Vice Chancellor of Kabarak University during our formative years in K.M.T.C, Nakuru were not in vain or so I believe. I thank my field work research team members who are too many to enumerate. However, on behalf of the rest, I must mention my special appreciation for the clinical and laboratory assistance from Mr. Koech, Mr. Ambrose Lang'at, Mr. Lundu, and Mr. John Kweka (KEMRI) among many others associated with the DVBD-KEMRI/CDC-accredited research laboratory and the entire Kapsabet District Hospital and Kapkangani Health Centre. I thank Dr. Too, Mr. Julius Koech of Moi Teaching and Referral Hospital/AMPATH and Mr. Ben.Kiprono Koech for their tireless efforts in data analysis. I also thank all others who contributed directly or indirectly towards the completion of my work. Last but not least, I thank my employer, Moi University, for partial funding and other forms of support to do most of what I have managed to accomplish in my doctorate pursuit.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is one of the parasitic diseases responsible for high morbidity and mortality, especially among the children in Sub-Saharan Africa (WHO, 2008). As a result, there is still a lot of research on malaria aimed at alleviating the socioeconomic problems the disease has caused. Although all human beings are vulnerable, children aged five and below, pregnant mothers and the elderly are the most susceptible. Most of the people that are adversely affected by malaria live in Sub-Saharan Africa (Snow *et al*., 1999).

Despite the urgency of swift treatment of malaria that is sometimes mandatory, there is a need to overcome the rampant misdiagnosis (either over-diagnosis or under-diagnosis) of malaria that may partly be responsible for the high morbidity and mortality from malaria (WHO, 2006). This is because misdiagnosis may either lead to delayed treatment of malaria or other disease resembling or coexisting with malaria (Reyburn *et al*., 2004), with fatal consequences or complications that could otherwise have been averted. Furthermore, a lot of resources are wasted on account of over-diagnosis and subsequent presumptive and/or over-treatment of malaria or other illness resembling or coexisting with malaria. Despite this, there is very little evidence to guide decision-makers on the relative cost-effectiveness of presumptive treatment, malaria rapid diagnostic tests and microscopy diagnostic methods across epidemiological settings (Menge *et al*., 2008; Msellem *et al*., 2009). In this study presumptively treated malaria refers to malaria recorded as having been treated without laboratory confirmation or regardless of absence of malaria parasites from laboratory results.

All factors that hinder accurate malaria diagnosis have a bearing on the identification and interpretation of malaria parasitaemia during laboratory diagnosis. There is therefore a need to improve malaria diagnosis using the most appropriate clinical and laboratory methods.

1.2 Problem Statement

Most of the world's human population that is affected by malaria lives in Sub-Saharan Africa. Although some progress has been made in improving the available microscopic techniques; for example, the Centers for Disease Control (CDC) personnel have developed a portable, battery-powered light source for field microscopy that has proven very useful in field trials (CDC, 2006), there are still many problems to be solved in order to improve malaria diagnosis. The technically qualified personnel expected to implement the policy procedures to successfully diagnose and manage malaria have to gradually cultivate a positive attitude and approach towards the same. Evaluations of gains made and the possible need to re-visit approaches to problematic areas should also be included in the overall management of malaria. Unfortunately, peer-reviewed articles reporting independent evaluations of malaria rapid diagnostic tests are scanty for most commercially available MRDT today (WHO, 2006).

In many developing countries, microscopy is not reliable because the microscopists are insufficiently trained, supervised and are overworked, the microscopes and reagents are of poor quality, and often the supply of electricity is unreliable. This situation calls for introduction of other supportive but reliable methods of malaria diagnosis. Indeed, in a meta-analysis of community-based studies that use Polymerase Chain Reaction (PCR) detection of parasites, Okello *et al* (2009) showed that microscopy only detects about

50% of the malaria infections identified by PCR method. Conversely in non-endemic malaria countries, laboratory technicians are often unfamiliar with malaria and may miss to recognize the malaria parasites (Susi *et al*., 2005). Nevertheless, microscopic examination of blood smears remains the current 'gold standard' for malaria detection and differentiation of various malaria parasite species. Molecular diagnostic tools, such as those based on PCR, have 10–100 greater sensitivity compared to microscopy and have been used increasingly for assessing infection (Greenwood, 2002; Dal-Bianco *et al*., 2007; Babiker & Schneider, 2008). Although reverse transcription PCR methods for detection of gametocytes have documented higher rates of gametocyte infection than microscopy (Menegon *et al*., 2000; Maeno *et al*., 2008) they are expensive, and require standardized protocols and validation of the specific protocol used in a given trial. For these reasons, PCR is not generally accepted as the "gold standard" for malaria diagnosis (Hamer *et al*., 2002; Murray *et al*., 2008).

Since most of the contemporary established molecular methods were too expensive to be widely used in most rural health centres, an attempt to use the recommended rapid malaria diagnostic kits should still be made, in order to overcome the rampant misdiagnosis of malaria that may partly be responsible for the high morbidity and mortality from malaria. The World Health Organization recommends that in areas where two or more species of malaria parasites are common, only the parasitological method will permit a species diagnosis (WHO, 2006). The World Health Organization further recommended that in places with mixed malaria parasite species, especially in absence of microscopy method, rapid diagnostic tests that are sensitive to *Plasmodium falciparum*

should be encouraged (WHO, 2006), given that *P.falciparum* is known to be more dangerous, as far as acute malaria epidemics are concerned.

Idealy, the introduction of relatively new methods for malaria diagnosis, such as rapid diagnostic tests, should reduce the practice of treating malaria presumptively. However, formal analyses have not estimated the epidemiological and economic thresholds at which different diagnostic strategies are preferable. In health centres and dispensaries situated in remote places, patients cannot easily access medical services, unlike the situation in larger health institutions, usually situated in urban areas. Health workers in remote places need reliable methods of malaria diagnosis. It is from this perspective that malaria rapid diagnostic tests should be explored. There is an increase in the use of rapid diagnostic tests (RDTs) that are based on detection of parasite antigens (Msellem *et al*., 2009).

1.3 Justifications of the Study

The fact that serious malaria epidemics have periodically occurred within the highland regions of western Kenya (Chandy et al., 2009) is enough reason not to be complacent about prioritizing malaria diagnostic and prevention measures within the highland region of western Kenya. Furthermore a recent study in Tanzania demonstrated that use of insecticide-treated bed nets (ITNs) was found to be inconsistently protective against sub-microscopic carriage of malaria parasites (Manjurano *et al*., 2011). This scenario calls for more effective diagnosis of malaria in areas prone to malaria epidemics, such as western Kenya highlands. In other parts of the world where malaria is endemic, there is an increase in the use of rapid diagnostic tests (RDTs) that are based on detection of parasite antigens (Msellem *et al*., 2009). However, there is relatively scanty data available on test

performance of rapid diagnostic tests on children as regards the study of febrile illnesses (Gasser *et al*., 2005; Murray *et al*., 2008), especially in the highland regions of western Kenya.

Currently, there is also no data on the user-attitudes towards malaria rapid diagnostic tests by health professionals in western Kenya. Furthermore, the recommended independent evaluations of malaria rapid diagnostic (WHO, 2007) was lacking in the highland regions of western Kenya, prior to the time our study was about to begin. Since inception of malaria rapid diagnostic test products, many users faced many challenges. The challenges ranged from rapid introduction, withdrawal, and modification by their manufacturers, inconsistency in manufacturing standards, quality control problems, and variable product stability. There is need to establish reasons for failed implementation of malaria diagnosis using rapid diagnostic tests (Hamer et al., 2007; Reyburn et al., 2007) in many regions known to have malaria epidemics, including western Kenya highlands. Furthermore, the trust by health professionals on the introduction of rapid diagnostic tests has been generally low in some places (Reyburn et al., 2007). We need to know why this is the case.

At the time our study was being carried out, there were no formal studies in highland regions of western comparing malaria rapid diagnostic test sensitivities in plasma, whole blood and serum conducted in children with malaria. Once this is known, it will enhance the cost-effectiveness of malaria diagnosis using the MRDT.

Even if malaria is successfully eliminated from the highland regions, a potentially long-term problem to keep in mind is that the longer the time that the populations at these sites

are unexposed to malaria, the more susceptible they are to malaria epidemics, due to lowered immunity to malaria. Epidemics could occur if an increase in vector density occurs in conjunction with the arrival of infected persons or mosquitoes from an area of higher transmission of malaria to less affected areas. Since traveling from one place to another has generally become more efficient due to improved transport means, it is also possible to transport malaria parasites through vectors that might inadvertently be in vehicles such as cars, lorries, trains and the like. Malaria-infected individuals may also move quickly from a malaria-endemic region to a less-endemic region, thus spreading the malaria parasites to susceptible victims. Thus, effective anti-malaria drugs must consistently be available to treat any infected and symptomatic travelers or immigrants to the area. As malaria cases decrease, microscopists will also need to receive training to remain proficient in detection of malaria in blood smears (Chandy *et al*., 2009). This implies that irrespective of the outcome of any malaria intervention measures, efficient and cost-effective malaria diagnostic methods will always be required in places where malaria is endemic or seasonally occurs in epidemics; hence our study objectives are also justified for this reason.

1.4 Limitations of the study

During the time of the study, there were irregularities in the seasonal rainfall that affected many epidemic malaria-prone highland areas of western Kenya. As a result, there were prolonged dry spells that tended to inhibit the breeding of mosquitoes. The number of children who suffered from malaria during such dry spells was relatively smaller compared with the numbers usually seen during epidemics or during rainy seasons. Because of the interrupted rainfall patterns as a result of the dry period extending from

September 2009 to April 2010, a pattern that was repeated during September 2010 to April 2011, only one hundred and thirty two (132) children that fulfilled the criteria of the study were systematically sampled during the entire period of the data collection for the study. Furthermore, the study was conducted at a time when the the indoor spraying of households with residual insecticides had already been successfully initiated and implemented. This was done as part of the larger annual Indoor Residual Spray (IRS) programme aimed at interrupting malaria transmission by spraying inside residential houses with long-acting residual insecticides for prolonged periods. The limitations already mentioned therefore necessitated an extension of the period of data collection up to May, 2011. The principal investigator of the study therefore appreciated the written support from the supervisors and the Institutional Research Ethics Committee (IREC), which enabled the successful completion of the study.

1.5 Objectives of the study

1.5.1 General Objective of the Study

The study determined the specificity and sensitivity of Immunochromatographic malaria rapid diagnostic test with microscopy as the gold standard, malaria parasite densities and haemoglobin levels. The study also evaluated predominant malaria features in children (<12 years), the rapid diagnostic test training and user-attitudes among healthcare professionals and malaria treatment practices in selected rural health centres in the epidemic malaria-prone highland region of western Kenya.

1.5.2 Specific Primary Objectives of the Study

1 To determine the sensitivity and specificity of Immunochromatographic (ICT) malaria rapid diagnostic test, using microscopy as the gold standard.

2 To determine the training and user- attitudes of health professionals in the epidemic malaria-prone highland and malaria-endemic lowland regions of western Kenya, towards malaria diagnostic tests.

1.5.3 Specific Secondary Objectives of the Study

3 To determine the age distribution of children with predominantly elicited clinical features of malaria.

4 To determine malaria parasite densities, haemoglobin levels and their distribution among the children of various age groups in the study population (< 12 years of age).

1.6 Null Hypotheses of the study

(1) Immunochromatographic (ICT) rapid diagnostic test is not sensitive/specific to *Plasmodium falciparum* among children < 12 years of age ($p > 0.05$).

(2) There are no differences among the sensitivities of ICT malaria rapid diagnostic test on serum, whole blood and plasma for the detection of malaria ($p > 0.05$).

(3) There are no differences in training and user-attitudes among health professionals in western Kenya highlands and lowlands, towards malaria rapid diagnostic tests to support microscopy ($p > 0.05$).

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria Diagnosis and Management Practices

Globally, there were an estimated 881, 000 (ranging between 610,000 and 1, 212,000) malaria human deaths in 2006, of which 91% (801, 000) were in Africa and 85% were of children under 5 years of age (WHO, 2008). Hence the morbidity and mortality from malaria remains unacceptably high among children, since over 40% of the world's children live in malaria-endemic countries (Snow *et al*., 1999). A key to effective management of malaria is based on its prompt and accurate diagnosis. The global impact of malaria has spurred interest in developing diagnostic strategies that will be effective not only in resource-limited areas, where malaria has a substantial burden on society, but also in developed countries, where expertise in malaria diagnosis is often lacking (Reyburn *et al*., 2007).

Malaria in Kenya is no longer confined to the malaria-endemic lowlands where it remains endemic, but frequently occurs in epidemics within the epidemic malaria-prone highlands, causing high morbidity and mortality among the people not immune to malaria (Ngindu *et al*, 1989; Some, 1994; Bhattacharya & Manesh, 1998). One known reason for this scenario is global warming that may encourage mosquito breeding (Zell, 2004). Laboratory diagnosis is an important component of case management and control of malaria (WHO, 2006); hence microscopy is routinely used for malaria diagnosis and epidemiological studies (Menge *et al*., 2008; Msellem *et al*., 2009). However, microscopy has limitations due to the subjective nature and sensitivity of slide reading

and its time-consuming nature when carrying out studies involving a large number of individuals (Menge *et al*., 2008; Shekalaghe *et al*., 2007; Greenwood, 2002; Dal-Bianco *et al*., 2007). Inaccurate microscopy and symptomatic diagnosis of malaria occur frequently in most endemic countries (Amexo *et al*., 2004; English *et al*., 2004; Reyburn *et al*., 2004; Susi *et al*., 2005). This has been attributed to inadequate financial resources to support diagnostic services, insufficient skilled laboratory personnel and low reproducibility of laboratory results (Bates &S Maitland, 2006; Ishengoma *et al*., 2010; Kassu & Aseffa, 1999). In these countries with limited resources, clinical guidelines have been developed and recommended for symptomatic and differential diagnosis of malaria. Some of these guidelines have been reported to be highly sensitive in detecting malaria cases but their specificity is low because malaria symptoms are quite often similar to those of other febrile tropical diseases (Msellem *et al*., 2009). Malaria diagnosis based on clinical signs and low accuracy of malaria microscopy have resulted in over-diagnosis of malaria, which carries a risk of unnecessary use of antimalarial drugs (the main cause of parasite resistance) and masking other underlying causes of febrile illnesses (Reyburn *et al*., 2004). Likewise under-diagnosis of malaria may result in delayed treatment and progression into severe cases with fatal consequences. The use of clinical guidelines at low prevalence of malaria symptomatic diagnosis carries a low risk of missing malaria cases but is also associated with substantial over-prescription of anti-malarial drugs (Chandramohan *et al*., 2002). Although symptomatic diagnosis of malaria has been considered reasonable in resource-poor settings with high malaria transmission where laboratory infrastructure is inadequate (WHO, 2003), the contemporary level of misdiagnosis has been found to be unsustainable particularly after introduction of the

more expensive artemisinin-based combination drugs (Perkins & Bell, 2008; Zurovac *et al*., 2006) and more sophisticated diagnostic techniques such as flow cytometry (Wongchotigul *et al*., 2004) among others.

In those areas with adequate malaria diagnostic services, perceptions and practices of clinicians stand to be important barriers to effective utilization of laboratory results. Previous studies have shown that malaria diagnostic practices have a strong link to clinical and contextual factors where malaria is strongly promoted as a disease that could be easily diagnosed clinically (Chandler *et al*., 2008; Chandler *et al*., 2008).

It can be argued that, shortage of qualified laboratory personnel and inadequate quality assessment systems contribute significantly in eroding the confidence of clinicians in applying laboratory results (Petti *et al*., 2006). However, with optimal malaria laboratory testing, it has been recognized that the test results may remain underutilized in managing febrile illnesses (Barat *et al*., 1999; Reyburn *et al*., 2007).

The introduction of high-cost antimalarial drugs such as artemisinin-based combination therapy (ACT) is encouraging malaria-endemic countries in sub-Saharan Africa to reassess diagnostic practices. This is because artemisinin-based combination therapy is more expensive than previous antimalarial drugs, occasionally has more limited supply and is potentially more toxic (Barnish *et al*., 2004; Amexo *et al*., 2004; Bell *et al*., 2008). The traditional practice of treating malaria presumptively based on history of fever (O'Dempsey *et al*., 1993; Chandramohan *et al*., 2002) may not only prove expensive to most people but also, it will be a wastage of malaria drugs for the patients treated without malaria (Chandramohan *et al*., 2002; Brinkmann & Brinkmann, 1991; Amexo *et al*., 2004; Reyburn *et al*., 2004). In many settings, empirical treatment results in substantial

overuse of antimalarial drugs and delays the diagnosis of other febrile illnesses (Chandramohan *et al*., 2002; Ndyomugyenyi *et al*., 2007; Sowunmi & Akindele, 1993). Furthermore, overdiagnosis of malaria implies underdiagnosis and inappropriate treatment of non-malarial febrile illness, such as the potentially fata bacterial meningitis, some viral diseases and respiratory infections (Brent *et al*., 2006).

The World Health Organization has recommended that parasite-based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high-prevalence areas (WHO, 2005; WHO, 2006). The need to adhere to WHO guidelines that malaria should be considered even in the presence of a negative malaria test remains vital, until more research-based evidence is accumulated. Those who oppose the proposed change further reason that a switch from presumptive treatment to laboratory-confirmed diagnosis and treatment may seem urgent but needs to be carefully considered and planned (D'Acremont *et al*., 2007).

Research by Nyamongo (2002) showed that malaria intensity and its expected cost increased when patients were moved from one treatment option to another; hence the decision to change treatment options should be done carefully, especially when treating children. This precaution should be considered before the final decision on whether or not, to abandon the treatment of malaria on basis of presumptive diagnosis. It is because of such diagnostic challenges that the existing malaria diagnostic methods should either be improved or supported by other additional cost-effective diagnostic methods. To partly resolve this diagnosis challenge, our study compared the sensitivity of immunochromatographic rapid diagnostic test (ICT) with specific clinical features of malaria and also correlated the haemoglobin levels with malaria parasite densities among

children with malaria. The introduction of malaria rapid diagnostic tests in recent years has triggered much interest among scientists who are researching on malaria. However, majority of published studies have evaluated malaria rapid diagnostic tests (MRDT) in health care settings where trained personnel performed the assay and microscopy.

The proponents of the change debate argue that there is accumulating documentary evidence showing that rapid diagnostic test performance is high, even when used in routine practice (Hopkins *et al*., 2008; Abeku *et al*., 2008). One major problem that may undermine success of the new-generation rapid diagnostic tests is the poor preparation of blood smears for examination (McMorrow *et al*., 2008). Hence it may no longer be necessary to provide treatment based on presumptive diagnosis. The first reason given by proponents is the significant reduction of fever, *P. falciparum* transmission, morbidity and mortality from malaria, even in areas previously considered highly endemic to malaria (WHO, 2008; Guerra *et al*., 2008). The implication of the reduction is that the lower the transmission, the lower the probability that a fever episode will be due to malaria. Secondly, new and reliable rapid diagnostic tests are now available to allow proper diagnosis of malaria at all levels of the health system. Proponents further say that the new- generation rapid diagnostic tests are reliable, easy-to-use, and cheap. They have good sensitivity, require minimal training and equipment, and retain accuracy even after extensive storage, including the real conditions found within the tropics. Some evidence has shown that the strategy based on documented diagnosis is safe (Njama-Meya *et al*., 2007; Lemma *et al*., 2008) even in uncontrolled settings and across age groups, according to a study done in Kenya (Zurovac *et al*., 2008).

Proponents also argue that the choice to treat all fever patients with anti-malarials leads to a huge drug wastage, which may potentially lead to unnecessary shortage of anti-malarials. Inappropriate use of anti-malarials (to treat viral or bacterial diseases that clinically resemble malaria) may also cause unnecessary adverse reactions, increased parasite resistance and loss of public confidence on the efficacy of artemisinin-based combination therapies (ACTs). Parasitological diagnosis and treatment with ACTs according to test results versus presumptive treatment with ACTs is cost-effective in all current malaria-endemic situations, provided the laboratory test results for malaria are considered (Lubell *et al*., 2007; Shillcutt *et al*., 2008). Proponents argue further that presently, the risk of missing to diagnose malaria due to a false-negative test is substantially smaller than the risk of the patient dying from another severe disease as the clinician remains focused on treating malaria. The risk of a false-negative test and its potential consequences have been evaluated thoroughly in Uganda, using microscopy (Njama-Meya *et al*., 2007) and in Tanzania, using rapid diagnostic tests and the safety of not treating malaria-negative children confirmed.

The two schools of thought, either proposing or opposing the change from assumptive fever to laboratory-based diagnosis of malaria, seem to have come close to agreeing that further strengthening of diagnosis and treatment practices under the proposed change is required, irrespective of the age group differences (Zurovac *et al*., 2008). Whatever the outcome of the on-going debate, it should be considered that large-scale distribution of rapid diagnostic tests is a great challenge that requires theoretical and practical training, regular supervision, and sustained financial mechanisms to ensure constant availability. It is crucial that quality control is implemented at all steps. Should rapid diagnostic tests be

introduced on a large scale, clinicians will need to be trained to manage the "negative syndrome" (patients with a negative malaria test). This will be challenging to them, especially considering their previously upheld notion that all patients with fever should be treated for malaria.

Since published research findings from Kenya to support either side of the on-going debate is scanty, there is an urgent need to conduct such research in Kenya because the country has high morbidity and mortality from malaria, especially among children (Ministry of Health (Kenya) National Malaria Strategy: 2001-2010); hence one reason for the current proposed study. The resolutions of the debate therefore will have a bearing on the affordability of malaria treatment in resource-poor communities of the world, which coincidentally happen to be the same populous communities of Sub-Saharan Africa. Although many countries in Africa are already embracing the shift from malaria treatment based on presumptive diagnosis to one that is based on laboratory diagnosis, some scientists are reluctant to totally abandon the treatment of malaria on the basis of presumptive diagnosis; they argue that since malaria has long been the leading cause of child mortality in malaria-endemic areas of Sub-Saharan Africa, any fever episode in children from malaria-endemic areas should be treated for malaria, if no other cause of fever cannot be identified, in order to save lives (English *et al*., 2009). Furthermore, presumptive treatment is preferred in some places because light microscopy, which for decades has been the standard for malaria diagnosis, may still be inaccessible due to lack of laboratory infrastructure and technical expertise it requires (Moerman *et al*., 2003; Bloland *et al*., 2003). Even when accessible, malaria microscopy results are not trusted by some practicing clinical practitioners, according to a resent research in Tanzania

(Yahya *et al*., 2011). The problem of malaria diagnosis is therefore not only in Kenya but also in the neighbouring Tanzania and possibly other regions of the world.

2.2 Challenges of Malaria Rapid Diagnostic Tests and Microscopy

Malaria rapid diagnostic tests (MRDT) represent an evolving technology that can be applicable in a spectrum of settings extending from diagnosis of imported malaria in tertiary hospitals in regions where malaria is not endemic, to remote health care clinics without clinical laboratories. Rapid diagnostic tests (MRDT) are increasingly becoming useful in malaria diagnosis. Some of the common MRDT techniques include: the Quantitative Buffy Coat (QBC) method (Bhandari *et al*., 2008), the OptiMAL (Tagbor *et al*., 2008; Zerpa *et al*., 2008) Immunochromatographic test (Ratsimbasoa *et al*., 2008), Para-HIT-f (McMorrow *et al*., 2008), ParaScreen (Endeshaw *et al*., 2008), SD Bioline (Lee *et al*., 2008), Paracheck (Harvey *et al*., 2008), and molecular diagnostic methods, such as polymerase chain reaction (Holland & Kiechle, 2005; Vo *et al*., 2007).

Most MRDT products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some rapid diagnostic tests detect *P. falciparum* specific and pan-specific antigens (aldolase or pan-malaria pLDH), and distinguish non-*P. falciparum* infections from mixed malaria infections. To alleviate the problem of inexperience, rapid diagnostic tools (MRDT) based on the dipstick principle for the detection of plasmodial histidine-rich protein 2 (HRP-2) and parasite-specific lactate dehydrogenase (pLDH), respectively, have been availed for the qualitative detection of *P. falciparum* malaria (Molyneux & Fox, 1993). The MRDT that currently appear most suitable for clinical use identify histidine-rich protein 2 (HRP2) or *Plasmodium* lactate dehydrogenase (pLDH). HRP2- based tests have been available in

various formats for several years, have shown good sensitivity in a variety of field settings, and they are increasingly recommended for use in settings where reliable microscopy is not available (Rafael *et al*., 2006).

Malaria antigens currently used as diagnostic targets are either specific to a *Plasmodium* species or conserved across the human malarias. *P. falciparum*-specific monoclonal antibodies have been developed for histidine-rich protein 2 (HRP-2) and *P. falciparum* lactate dehydrogenase, while *P. vivax*-specific monoclonal antibodies have undergone limited evaluation (Bell and Peeling, 2006; Forney *et al*., 2003). Targets conserved across all human malarias (pan-*Plasmodium* antigens) have been identified on *Plasmodium* lactate dehydrogenase (PLDH) and aldolase enzymes (Brown *et al*., 2004; Forney *et al*., 2003; Makler & Hinrichs, 1993; Makler *et al*., 1998). The first antigen used in a commercial assay was HRP-2, a water-soluble protein unique to *Plasmodium falciparum*, which is localized in the parasite cytoplasm and on the parasitized erythrocyte membrane (Howard *et al*., 1986; Shiff *et al*., 1993). The HRP-2 concentration increases as the parasite develops from the ring stage to the late trophozoite. This antigen readily diffuses into the plasma (Howard *et al*., 1986; Rock *et al*., 1987). It is found predominantly in the asexual stages but is also found in young *P. falciparum* gametocytes (Hayward *et al*., 2000; Tjitra *et al*., 2001). It can be detected at lower levels of parasitemia than the pan-malarial antigens such as aldolase (Gasser *et al*., 2005; Richter *et al*., 2004). Many different monoclonal antibodies, both immunoglobulin G (IgG) and IgM, have been raised against this antigen and have been employed on different MRDT. Variants that escape monoclonal recognition have been identified and may be responsible for false negative tests (Baker *et al*., 2005; Lee *et al*., 2006). In an assessment of HRP-2

in parasites obtained from 19 countries, an extensive level of diversity of HRP-2 sequences was observed, prompting a prediction that only 84% of *P. falciparum* infections with low parasite densities (250 parasites/µl) could be detected in the Asia-Pacific region (Lee et al., 2006). Parasite enzymes comprise the other primary antigen diagnostic targets. PLDH, the terminal enzyme in the malaria parasite's glycolytic pathway, is also an antigen target for detection of sexual and asexual malaria parasites. Monoclonal antibodies have now been developed that can target a conserved element of PLDH on all human malaria species (panmalarial) or specific regions unique to *P. falciparum* or *P. vivax*. Aldolase, a key enzyme in the glycosis pathway in malaria parasites, is also well conserved across all human-specific species of *Plasmodium* and is used as a panmalarial antigen target (Genrich *et al*., 2007; Lee *et al*., 2006). Other antigens have been recognized as possible components of future diagnostic tests, but no evaluations of *P. ovale*- or *P. malariae*-specific antigens have been published (Forney *et al*., 2003; Nyame *et al*., 2004; Suh *et al*., 2003).

MRDT performance for diagnosis of malaria has been reported as excellent (Kyabayinze *et al*., 2008; Ratsimbasoa *et al*., 2008; Endeshaw *et al*., 2008; Harvey *et al*., 2008; Chilton *et al*., 2006; Doderer *et al*., 2007). However, some reports from remote malaria-endemic areas have shown wide variations in the sensitivity of MRDT (Murray *et al*., 2008). Overall, MRDT appears a highly valuable, rapid malaria-diagnostic tool for health professionals; however they must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment. Few studies have compared MRDT performance among areas with different levels of endemicity and populations, and these studies have not identified consistent associations between malaria

transmission rate andMRDT performances (Mharakurwa *et al*., 1997). In addition, many prior studies have used light microscopy results as the gold standard when measuring the diagnostic accuracy of MRDT (Cooke *et al*., 1999; Guthmann *et al*., 2002; Mboera *et al*., 2006). This approach may underestimate the performance of MRDT, because MRDT may detect parasites below the limits of light microscopy. Other studies have highlighted the significance of subpatent parasitemia, both clinically and as a reservoir for transmission (Bell *et al*., 2005; Giha *et al*., 2005; Mockenhaupt *et al*., 2006; Mens *et al*., 2007; Shekalaghe *et al*., 2007). Historically, malaria prevalence surveys have been conducted using field microscopy as the diagnostic method. These studies typically suffer from microscopy's lack of sensitivity for detecting the low parasitemias of asymptomatic carriers (Coleman *et al*., 2002; Mens *et al*., 2007; Shekalaghe *et al*., 2007). While MRDT would reduce logistical challenges in these studies, their parasitemia threshold of detection does not appear to be sufficiently low to be useful for asymptomatic screening (Coleman *et al*., 2006; Mens *et al*., 2007; Shekalaghe *et al*., 2007).

However, a disadvantage of HRP2-based rapid diagnostic test assays is the persistence of detectable circulating antigen for up to several weeks after parasites have been eradicated (Tjitra *et al*., 2001; Singh & Shukla, 2002; Mayxay *et al*., 2001; Swarthout *et al*., 2007). Persistent antigenemia may limit the usefulness of HRP2-based assays in areas of intense malaria transmission, where positive tests may commonly be the result of prior infections that are no longer clinically relevant. Aldolase and PLDH rapidly fall to undetectable levels after initiation of effective therapy, but all of these antigens are expressed in gametocytes, which may appear after the clinical infection is cleared (Mueller *et al*., 2007). Therefore, none of these assays is useful for monitoring the response to treatment.

Microscopy remains the test of choice for this purpose. PLDH-based MRDT appear to be less sensitive than tests that detect HRP2, but they are more specific, aspLDHis rapidly cleared from the bloodstream and becomes undetectable at about the same time blood smears become negative after antimalarial therapy (Piper *et al*., 1999; Moody *et al*., 2000; Oduola *et al*., 1997). HRP2- and pLDHbased tests also differ in the parasite species they detect: the HRP2 test detects only *Plasmodium falciparum,* whereas the pLDH test detects all 4 species that cause human malaria. Although MRDT clearly show promise as new diagnostic tools for Africa, it is not clear where MRDT should replace presumptive therapy or light microscopy, nor is it clear which MRDT is most appropriate for different epidemiological settings. The use of Malaria Rapid diagnostic tests (MRDT) does not eliminate the need for malaria microscopy. MRDT may not be able to detect some infections with lower numbers of malaria parasites circulating in the patient's bloodstream; hence MRDT provide no quantification of parasite density. For this reason MRDT are not useful in screening malaria from prospective blood donors (Seed *et al*., 2005).

Immunochromatographic test (ICT) detects malaria antigens in patient blood. However, OptiMAL and Immunochromatographic (ICT) rapid diagnostic tests have the disadvantages of being specific to *P. falciparum,*cannot be used for drug monitoring owing to persistent levels of HRP-2 for about 15 days following treatment and the fact that the tests cannot identify 'carriers', due to absence of HRP-2 in gametocytes (USAID, 2008).

Another rapid diagnostic method for malaria that has been used, especially on travelers that had returned from malaria-endemic areas, is the Quantitative Buffy Coat (QBC) test.

The QBC technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis (Clendennen *et al*., 1995). The QBC technique has been shown to be a rapid and sensitive test for diagnosis of malaria in numerous laboratory settings (Bhandari *et al*., 2008; Pornsilapatip *et al*., 1990; Barman, 2003; Adeoye and Nga, 2007). The QBC method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by epi-fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope (Chotivanich *et al*., 2006). Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange.

Although the QBC technique is simple, reliable, and user-friendly, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites. While it enhances sensitivity for *P. falciparum*, QBC reduces sensitivity for non-*P.falciparum* species and decreases specificity due to staining of leukocyte DNA. It has been shown that acridine orange is the preferred diagnostic method (over light microscopy and immunochromatographic tests) in the context of epidemiologic studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitemia (Ochola *et al*., 2006). Nowadays, portable fluorescent microscopes using light emitting diode (LED) technology, and pre-prepared glass slides with fluorescent reagent to label parasites, are available commercially. In a retrospective study of 399 blood sera samples by Durand et al., (2005), the Now ICT Malaria rapid test was compared with the quantitative buffy

coat (QBC) test and microbiological examination of thin blood films. Compared with the QBC test and thin blood films, the Now ICT Malaria test had sensitivity and specificity values of 96.4% and 97%, respectively, for the detection of pure *P. falciparum* infection. A negative predictive value of 99.4% allows this test to be included in diagnostic strategies for patients presenting with clinical suspicion of malaria. Two false-negative results were associated with low levels of parasitaemia in the specimens. Thus, use of the Now Malaria test alone to detect *P. falciparum* infection in non-endemic countries could lead to misdiagnosis of malaria. This rapid diagnostic test should therefore be performed in association with another prompt traditional method such as examination of thin blood films.

The MRDT require limited or no need for instrumentation and therefore can be performed at numerous peripheral health institutions. In some cases, rapid diagnostic tests are more accurate than the existing reference-level laboratory tests (USAID, 2008). Malaria MRDT are also a valuable resource for diagnosis of malaria in travelers and military personnel presenting to medical providers unfamiliar with malaria clinical diagnosis.

Although most MRDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria (Park *et al*., 2006; Lee *et al*., 2008; Kim *et al*., 2008). There are other important factors to be considered in the assessment of malaria rapid diagnostic tests. Various studies have shown that HRP2 MRDT remain positive from 6 to 31 days following radical treatment (Beadle, 1994; Uguen *et al.,* 1995*;* Karbwang *et al.,* 1996; Tjitra *et al.,* 2001). Sensitivity of MRDT can be adversely affected by concentration of the malaria parasites. The World

Health Organization (WHO) has recommended a minimal standard of 95% sensitivity for *P.falciparum* densities of 100/µl and a specificity of 95% (Bell & Peeling, 2006; WHO, 2006). While this degree of sensitivity may be possible for microscopy in high-quality reference laboratories, it is rarely achievable in hospital laboratories in malaria-endemic countries. The use of a decision tree model to evaluate the impact of introducing MRDT for the diagnosis of febrile children under the age of 5 years in sub-Saharan Africa not only demonstrated that an MRDT with 95% sensitivity for parasitemia of 500/µl and 95% specificity could avert over 100,000 malaria-related deaths, but it also cut down on the cost of treating malaria by avoiding about 400 million unnecessary treatments (Rafael *et al*., 2006).

In most of sub-Saharan Africa where malaria infections occur predominantly or solely with *P. falciparum*, use of an assay that detects *P. falciparum* alone may be clinically sufficient and more cost-effective. However, in places where malaria patients may be co-infected with another *Plasmodium* species or where relapses occur, additional treatment with primaquine may be required (Snounou & White, 2004). In situations like this, resources and the importance of detecting the other species will influence the most suitable type of MRDT that will eventually be chosen (WHO, 2006).

Malaria rapid diagnostic tests have been used experimentally for many years, initially in Thailand (Banchongaksorn *et al*., 1996; Stephens *et al*., 1999; Imwong *et al*., 2008), the United Republic of Tanzania (Shiff *et al*., 1994) and even in the United Kingdom (Chilton *et al*., 2006). Since the World Health Organization recognized the urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for determining the presence of malaria parasites, to overcome the deficiencies of light microscopy,

numerous new malaria-diagnostic techniques have been developed (WHO, 1996). Around 2008, 86 malaria rapid diagnostic tests were available from 28 different manufacturers (WHO, 2008). This, in turn, has led to an increase in the use of rapid diagnostic techniques (MRDT) for malaria, which are fast and easy to perform, and do not require electricity or specific equipment (Bell & Peeling, 2006). Furthermore, the tests require minimal training, no refrigeration and have a long shelf-life of one to two years at ambient temperatures; hence no need for refrigeration or cold chain (WHO, 2006). Misinterpretation of MRDT results may occur at low parasite densities, when there is poor lighting or when the viewer has poor visual acuity. Inadequate blood volume than instructed may reduce the antigens, while excess blood volume tends to inhibit clearance of blood stains (Murray *et al*., 2008). All these adverse factors demand the engagement of well-trained field workers to handle MRDT. Persistent antigenaemia, 14 days after parasite clearance, was detected by HRP2 MRDT in 10% of patients in the United Republic of Tanzania *(*Shiff *et al*., 1993). In a Kenyan study, of 134 children who were positive to the same HRP2-detecting test, 11.9% were still positive on the sixth day following radical treatment with quinine and doxycycline *(*Beadle, 1994). It has been suggested that the presence of *P. falciparum* gametocytes is responsible for some false-positive test results in *P.falciparum* infections (Tjitra *et al*., 2001;Tjitra *et al.*,1999; Guthmann *et al*., 2002), although one person had persistent HRP2 antigenaemia for 31 days following artemisinin combination therapy which had cleared all gametocytes (Tjitra *et al*., 2001). A further study found no significant association between persistent antigenaemia and gametocytaemia *(*Iqbal *et al*., 2004). In a trial designed to determine the persistence of *P. falciparum* antigens after successful treatment *(*Iqbal *et al*., 2004), it was

found that the pLDH-detecting test was more specific than the HRP2-detecting test for monitoring responses to anti-malarial therapy, since pLDH activity offers a good correlation with the presence of viable parasites and its half-life is shorter than that of HRP2 when parasitaemia is cleared. In the past, the cross-reaction between rheumatoid factor and HRP2 antigen contributed to false-positive results (Laferi *et al*., 1997; Bartoloni, 1998; Iqbal *et al*., 2000). This problem is thought to be less common with currently available tests (Craig *et al*., 2002). Now, the most common cause of false-positives is probably back-flow, resulting from the test results being read after the time recommended by the manufacturer. False-positive results may also be caused by the presence of heterophile antibodies, e.g. anti-mouse antibodies, but more research is needed in this area (Moody, personal communication). A false-negative result may have far more serious consequences, such as the patient's not receiving any antimalarial treatment or being given inappropriate medicines. In the United Republic of Tanzania, it was noted that false-negatives occurred in cases with low-level parasitaemia; most such cases had < 40 parasites/µl blood (Shiff *et al*., 1994). In Uganda it was shown that sensitivities were significantly lower when parasitaemia was 100 parasites/µl or less, than when it exceeded 100 parasites/µl (Guthmann *et al*., 2002). In a study carried out in Malawi (Mankhambo *et al*., 2002), the pLDH-based MRDT had a low sensitivity for the detection of placental *P. falciparum* infection and was no more sensitive than conventional microscopy for the detection of parasites in peripheral blood. In a more recent study in Nigeria, a pLDH-based test detected less than half as many peripheral parasitaemias in pregnant women as microscopy, probably because of low parasitaemia ((Tavrow *et al*., 2000). Although the International community has recognized that MRDT

are being used at almost every level of the health care system, the World Health Organization (WHO) has recognized that lack of quality control for diagnostic devices in the marketplace, including MRDT, has hampered the assessment of the best implementation practices in the public health infrastructure (Bell and Peeling, 2006). Placental malaria infection detected by MRDT using peripheral blood was notably associated with a lower median birth weight (Singer *et al*., 2004). The ability to detect placental infection by antigen detection when microscopy does not identify parasitemia could have a significant impact on material and fetal health care. The implications of persistent HRP-2 antigenemia for up to a month after successful therapy are unclear in fetal health care setting. The key to gaining this improvement in outcomes was the much wider availability of an MRDT leading to a marked increase in access to the population. The potential for MRDT to give false-negative results in testing samples with low but clinically relevant levels of parasitemia is noted above. The improved sensitivity of MRDT as antigenemia and, in rough proportion, parasitemia increase suggests that MRDT using blood samples obtained serially over 24 to 48 h might permit diagnosis even for patients whose initial test results were negative. This model of serial testing has long been used for microscopic examination of blood smears from patients suspected to have malaria even after a negative initial blood smear examination. Even with such serial testing using MRDT, however, expert microscopy would still be helpful to determine the species and the level of parasitemia (The Malaria Working Party of the General Haematology Task Force of the British Committee for Standards in Haematology, 1997). Malaria rapid diagnostic tests are increasingly being considered for routine use in many African countries (Lubell *et al*., 2008). From their introduction, these products suffered

from rapid introduction, withdrawal, and modification by their manufacturers, inconsistency in manufacturing standards, quality control problems, and variable product stability (Shiff *et al*., 1993; Mason *et al*., 2002). Due to the effect of sequestration of blood-stage *Plasmodium falciparum* parasites, observed parasitemia may not directly correlate with parasite antigen or biomass (Craig *et al*., 2002; Dondorp *et al*., 2005). In addition, parasite prevalence in the community affects the positive and negative predictive values of the test. Prior therapy and effectiveness of therapy vary between patients and can affect trial results because some antigens persist in blood for weeks after therapy, while others clear within a few days (Iqbal *et al*., 2004; Swarthout *et al*., 2007). Polymorphisms in the target antigen(s) from the parasite can also influence the results obtained from the MRDT (Joshi *et al*., 2003; Baker *et al*., 2005; Lee *et al*., 2006). Also, antigen production can vary between stages of the parasite life cycle and among parasite strains, and circulating gametocytes may produce the target antigen, even after therapy that has eradicated the asexual blood-stage parasites (Dondorp *et al*., 2005; Tjitra *et al*., 2001). Patient co-morbidities may also influence the results, as rheumatoid factor heterophilic antibodies can result in false-positive results for some patients (Craig *et al*., 2002; Moody *et al*., 2002). Clinical history from study subjects should be elicited, as pre-treatment with antimalarials has an impact on parasitemia but may be associated with lingering antigen. Aldolase and PLDH are rapidly cleared after effective therapy, while HRP-2 antigenemia may persist for longer than a month. All current antigen assays may revert to positive if gametocytes subsequently appear in the bloodstream (Miller *et al*., 2001; Mueller *et al*., 2007; Swarthout *et al*., 2007). This is important because not all therapeutic regimens, including chloroquine and quinine, are effective at eradicating

gametocytes, and some, especially sulfa-containing regimens, may actually induce gametocytemia (Puta & Manyando, 1997; Sokhna *et al*., 2001). Persistent low-level parasitemia, including levels below the threshold of microscopic detection, may also produce positive antigen test results (Bell *et al*., 2005).

Evaluations of various rapid diagnostic tests have also been done in several countries, but difficulties of procurement, distribution, transport and storage need to be addressed (Guthmann *et al*., 2002). Unfortunately, peer-reviewed articles reporting independent evaluations are scanty for most commercially available MRDT. The impact of the blood source (mixed-capillary sample obtained by finger stick versus venous sample obtained by phlebotomy) on the ability to detect circulating parasites and antigen has been evaluated rigorously, with no significant impact on HRP-2-based and aldolase-based test performance observed. Given the demonstrated potential for inconsistency among microscopists (Durrheim *et al*., 1997; Kachur *et al*., 1998), all smears should be interpreted by more than one microscopist blinded to both the MRDT results and the findings of the other microscopist(s), with a defined system to evaluate discordant results between the microscopists. Experts should be selected based upon predetermined qualification standards (Ohrt *et al*., 2007). Test performance characteristics are influenced by transport and storage of the diagnostic kits. These factors should be reported, particularly for evaluations of MRDT conducted under field conditions (Chiodini *et al*., 2007; Leke *et al*., 1999). Data on acceptance and test performance for self-diagnosis by travelers is limited, but as a proof of concept, Behrens & Whitty (2000) evaluated the ICT Malaria Pf test (an HRP-2-only test which is no longer available; ICT Diagnostics, Sydney, Australia) on 153 symptomatic volunteers who presented to the

Hospital of Tropical Diseases in London, United Kingdom. In a study among travelers presenting with fever in Kenya, only 68% of persons were able to perform and interpret the kit accurately, with 10 of 11 with malaria failing to diagnose themselves correctly (Jelinek *et al.*, 1999). Some studies have alluded to difficulties experienced by travelers in following the instructions, interpreting the results, and obtaining blood. Training and practice with good visual aids prior to travel were recommended. More data are clearly needed before any recommendations can be made concerning such self- or buddy testing with MRDT (Trachsler *et al*., 1999).

Studies assessing perception of patients or care-takers (Chandler et al., 2008; Tarimo *et al.*, 2000) and clinicians (Polage *et al.*, 2006) with respect to malaria diagnosis have elaborated among other things two important scenarios; that patients prefer laboratory testing before anti-malarial drug prescription and sometimes malaria laboratory results may have little influence in prescription practices by clinicians. The reasons behind unde-rutilization of malaria laboratory results by clinicians need to be investigated and addressed accordingly in order to build a culture of rational management of malaria (Bell & Peeling, 2006; WHO, 2000). The problems associated with MRDTs led to the introduction of guidelines outlined by the WHO to assess the utility of MRDT (Bell & Peeling, 2006; WHO, 2004; WHO, 2006).

Two meta-analyses have clearly shown that the performance of rapid diagnostic tests is comparable to that of expert microscopy (Ochola *et al*., 2006).

Over-diagnosis of malaria mainly on the basis of fever is still widely practiced; hence the risk of missing the diagnosis of fever due to non-malarial causes remains real. It is not also easy to intervene on the clinicians' management attitude towards febrile illnesses,

according to a study by Reyburn *et al*., (2007). It is therefore crucial that specific clinical features that are more closely associated with malaria are identified, in conjunction with the use of laboratory tests. Most of the studies carried out in the past have emphasized on the importance of malaria rapid diagnostic tests but the incorporation of clinical features alongside the use of rapid diagnostic tests has been recommended in most of the past researches. However, the specific clinical features are not clearly stated in the various past studies. Furthermore, the algorithms promoted by the Integrated Management of Children Illness (IMCI) for the clinical management of febrile children do not differentiate between malarial and non-malarial fevers. Although the IMCI strategy has been shown to be cost-effective in child health care *(*Goodman *et al*., 2000), probable over-diagnosis of malaria is among the problems constraining its effectiveness *(*Nicoll, 2000*)*. Towards filling this knowledge gap, one objective of the current study was to compare the clinical diagnosis of malaria and the diagnosis using malaria rapid diagnostic tests.

The requirements for malaria rapid diagnostic tests vary depending on malaria local epidemiology and the goals of a malaria control programme, as far as their performance and operational characteristics are concerned (Bell & Peeling, 2006; WHO, 1999). The epidemiology of malaria in the study population can influence the results of field trials (Bell *et al*., 2005; Swarthout *et al*., 2007). Hence there is a need to evaluate the factors that influence the use of specific MRDT in specific geographical regions, in comparison to other regions. The choice of the correct MRDT for use in malaria control and treatment programmes should be influenced by the epidemiology of malaria in the area to be served; hence it is clear that more studies on the effectiveness of MRDT are needed.

The study therefore aimed at overcoming this constraint by determining the specific clinical features that are useful in malaria diagnosis in children aged twelve and below, within the epidemic malaria-prone highland set-up of western Kenya.

2.3 Challenges of presumptive and laboratory diagnosis of malaria

In areas of Sub-Saharan Africa with high-malaria transmission, the distinction between the clinical disease of malaria and malaria parasitemia is especially difficult. Persons may present with a wide variety of other fever-inducing diseases accompanied by a parasitemia that is not related to the presenting symptoms (Taylor *et al*., 2004). Hence this is a major challenge for clinicians working in Sub-Saharan Africa, where clinicians tend to diagnose most febrile illnesses as malaria, especially if microscopy is not available for confirmation. However, in most resource-rich countries where malaria is rare, malaria diagnosis may also be missed by inexperienced clinicians. In either case, misdiagnosis of malaria is a frequent mistake that can have deadly consequences (Duffy & Fried, 2005; Reyburn *et al*., 2004; Brent *et al*., 2006; Berkley *et al*., 2005; Bhandari *et a*l., 2008). The overlapping of malaria symptoms with other tropical diseases also impairs diagnostic specificity, which can promote the indiscriminate use of anti-malarials and compromise the quality of care for patients with non-malarial fevers in endemic areas (Berkley *et al*., 2005; Mwangi *et al*., 2005; Reyburn *et al*., 2004; McMorrow *et al*., 2008). Unfortunately and regrettably so, malaria control in tropical Africa has principally been based on the presumptive treatment of fever using anti-malarial drugs (Chandramohan *et al*., 2002; Trape *et al*, 2002).

Historical strategies to diagnose malaria range from basic empirical clinical diagnostic clinical features to examination of stained blood smears by light microscopy. Although

empirical clinical diagnosis remains the most common method to diagnose malaria in many regions, the accuracy of this strategy is poor, even in countries of endemicity with high malaria incidence rates. The symptom complex of malaria overlaps with those of many other tropical diseases, and co-infections commonly occur (Schellenberg *et al*., 1994; Luxemburger *et al*., 1998; Chandramohan *et al*., 2002; Peters *et al*., 2004; Hamer *et al*., 2007; Bell *et al*., 2008).

A study by Ndyomugyenyi *et al*., (2007) found that malaria mainly presented with joint pains, headache, vomiting and abdominal pains; however, due to low prevalence of malaria, the predictive values of individual signs alone or in combination, were poor. Hence the study recommended that in low transmission areas, more attention needs to be given to differential diagnosis of febrile illnesses and that rapid diagnostic tests are needed so as to help clinicians to limit the prescription of ACTS to the patients who really need them. Perhaps this recommendation should be heeded in western Kenya highland regions.

The management of malaria, based on presumptive diagnosis has in the past been supported by many, including the World Health Organization. The World Health Organization (WHO) then advised that presumptive diagnosis should be the basis for first-line treatment of uncomplicated malaria in places where a parasitological test is not possible. However, due to the introduction of expensive anti-malarial drugs, such as the artemisinin-based combination therapy (ACT), many countries in Sub-Saharan Africa have had to reassess their diagnostic practices. Hence, the current tentative recommendation by WHO is that the parasite-based diagnosis should be used in all cases

of suspected malaria with the exception of among other situations, children in high-malaria prevalence areas (WHO, 2008).

In the past, an attempt to provide clinical features for the diagnosis of the common childhood illnesses has been made in various set-ups. One such algorithm has been provided by the Integrated Management of Children Illness (IMCI), which enables minimally trained healthcare providers in the developing world, who are exposed to inappropriate equipment for laboratory diagnosis of malaria among other illnesses (Tarimo *et al*., 2001). Research on IMCI use in African settings for malaria diagnosis by minimally trained healthcare personnel, compared with a fully trained pediatrician with access to laboratory support, showed very low specificity (0-9%) but 100% sensitivity (Perkins *et al*., 1997; Weber *et al*., 1997). This lack of specificity revealed the perils of distinguishing malaria from other causes of fever in children on clinical grounds alone. Another study showed that use of the IMCI clinical algorithm resulted in 30% over-diagnosis of malaria (Tarimo *et al*., 2001). The conclusion from the study was that the accuracy of malaria diagnosis can be greatly enhanced by combining clinical-and parasite-based findings (Kyabayinze *et al*., 2008).

During a study carried out in Madang, Papua New Guinea, the role of MRDT in discriminating malaria from other causes of fever was evaluated and compared with microscopy, the Integrated Management of Childhood Illnesses (IMCI) algorithm, and routine clinical diagnosis. The algorithm promoted by IMCI for the clinical management of febrile children does not differentiate between malarial and non-malarial fevers. According to IMCI guidelines, a child is classified as having “malaria” if he or she has a recent history of fever or feels hot or has an axillary temperature of 37.5 °C or above

(Smith *et al*., 1994). Although the IMCI strategy has been shown to be cost-effective in child health care *(*Goodman *et al*., 2000).The probable over-diagnosis of malaria is among the problems constraining its effectiveness (Nicoll, 2000). A total of 1058 children were enrolled in the study, and three MRDT (two different HRP2-detecting MRDT, and one MRDT detecting both HRP2 and pan-malaria aldolase) were evaluated. One conclusion of the study was that HRP2 tests should not be used to exclude malaria, and negative results should be considered also as "non-*P.falciparum*" malaria and treated accordingly. Another conclusion was that MRDT could be more effective in younger children (< 2 years of age) because of the lower prevalence of malaria in this age group. It was also concluded that MRDT may assist in the differential diagnosis of malaria and pneumonia, as defined by the IMCI guidelines. According to IMCI guidelines, a child is classified as having "pneumonia" if he or she has cough or difficult breathing with fast breathing (50 breaths per minute in children aged 2–11 months and 40 breaths per minute in children aged 1–5 years). The use of MRDT can be economically justified under specific conditions. The cost savings of malaria management with MRDT depend on a combination of factors such as the epidemiological pattern of malaria, the performance of the tests, the cost of the tests, and recommended treatment regimens. In areas of high malaria risk, MRDT seem to be more cost-effective in children under 3 years of age with lower malaria prevalence than they are in children aged 3–5 years. MRDT can be used by first-line health professionals at primary health care facilities. First-line health professionals completed the MRDT in less time than recommended by the manufacturers but still retained high sensitivity and specificity. The children aged five and below who live in areas of high malaria transmission are the most vulnerable age group, with the

highest malaria morbidity and mortality. They normally have the highest prevalence of malaria infection of all population groups. The risk of mortality in this age group, due to missed malaria diagnosis (false-negative result), may outweigh the risks and costs associated with over-treatment based on clinical diagnosis. In high-transmission settings, all under-5 children with a clinical suspicion of malaria should therefore be treated (WHO, 2006).

In areas of intense malaria transmission, the prevalence of parasitaemia is lower in individuals aged over five years; in areas of high malaria transmission, this group also experiences the lowest death rate due to malaria. Because of the high proportion of expected negative results and low risk of mortality in this group, treatment based on clinical signs and symptoms alone leads to considerable over-diagnosis and unnecessary consumption of antimalarials (Amexo *et al*., 2004) hence whenever malaria is suspected, a differential diagnosis should be considered, and treatment should be provided only after parasitological confirmation of the infection.

Timely laboratory diagnosis of malaria is therefore particularly important in its management and control (Hanscheid *et al*., 2001; WHO, 2006; Han *et al*., 2007). However, inaccurate microscopy and symptomatic diagnosis of malaria has in the past been frequently reported in most countries where malaria occurs in endemic proportions (Amexo *et al*., 2004; English *et al*., 2004; Reyburn *et al*., 2004). Nevertheless, it is also encouraging to note that the more intensified use of insecticide nets (ITNs) and research about the intermittent preventive iron and anti-malarial therapy (Egan *et al*., 2005) has drastically reduced the prevalence of malaria in the epidemic malaria-prone highland region of Western Kenya. Although an increase in frequency of the knockdown

resistance (*kdr*) mutation has been found in areas of high ITN use in western Kenya, there is as yet no evidence of phenotypic resistance to pyrethroid insecticides. The spread of this mutation and its impact on insecticide resistance continues to be monitored (Division of Malaria Control, Kenya, 2011).

2.4 Advantages of laboratory diagnostic techniques for malaria and their shortcomings

Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains (Warhurst & Williams, 1996). This method has changed very little since Laverran's original discovery of the malaria parasite, and improvements in staining techniques by Romanowsky in the late 1,800s. More than a century later, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films and thin blood films (for species' confirmation) remains the primary diagnostic (gold standard) for detection of malaria parasitaemia (Duffy & Fried, 2005; Bharti *et al*., 2007).

The sensitivity of the microscopic method can be excellent, with detection of malaria parasite densities as low as 5 to 10 parasites/µl of blood, which is approximately 0.0001% parasitemia (Murray *et al*., 2008). Malaria smears also provide a permanent record for quality assessment of the microscopy diagnosis (Murray *et al*., 2008). The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasitaemia levels, when parasites are sequestrated or when malaria parasite viability is impaired by chemotherapy. As a result of this shortcoming, highly sensitive detection of malaria cannot be achieved with Giemsa-stained thick and thin smears, for purposes of screening malaria from blood transfusion donors (Kitchen & Chiodini, 2006). Examination of serially obtained smears helps to overcome the

challenges posed by parasite sequestration and initially low parasite densities (Hanscheid, 1999).

These problems are exacerbated in regions where malaria is not endemic, as malaria microscopy is performed infrequently. Illustrating this problem, a study of 100 patients in Although the expert microscopist can detect between 5-10 parasites per microlitre of blood, the average microscopist detects only 50-100 parasites per microlitre of blood (Murray *et al*, 2008; Payne, 1988). This has probably resulted in under-estimating malaria infection rates, especially cases with low parasitemia and asymptomatic malaria. Furthermore, an individual microscopist's expertise in interpreting blood slides can diminish over time (Murray *et al*., 2008). The ability to maintain required levels of accuracy in malaria diagnostics expertise is problematic, especially in remote medical centers in countries where the disease is rarely seen (Ohrt *et al*., 2002). Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density is still challenging. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable (Erdman & Kain, 2008).

2.5 Malaria parasitaemia in children

Malaria parasitaemia prevalence has been studied in relation to different situations in various parts of the world. Parasitological, haematological, serological and entomological measures have in the past all demonstrated that malaria transmission intensity decreases with altitude (Drakeley *et al*., 2005; Drakeley *et al*., 2005; Carneiro *et al*., 2007). Various studies have also demonstrated that local factors that favour transmission will influence both microscopic and sub-microscopic parasite carriage. Similar findings have been

encountered in the highlands in Kenya (Ernst *et al*., 2006; Githeko *et al*., 2006) indicating that small differences in altitude and other factors can influence the ecological environments of mosquitoes.

It has also been previously documented that lack of parasitemia by microscopy and PCR in symptomatic and asymptomatic persons may be caused by seasonal variation common in highland areas and not by interruption of local transmission; hence this pauses an additional challenge in malaria diagnosis. In malaria endemic areas, asymptomatic malaria parasite carriers especially adults are not uncommon and, as potential gametocyte carriers, represent an important reservoir for malaria transmission (Alves *et al*., 2005). For many countries in Africa, historical data on malaria prevalence are available from surveys done by the Ministries of Health and are used for mapping and evaluating patterns of malaria endemicity and for implementing malaria control programmes within the public health sector (Najera, 1989; Omumbo *et al*., 1998). According to statistics published by the Division of Malaria Control, Kenya (2011), it is estimated that 60-70 per cent of the Kenyan land mass has a parasite prevalence of less than 5 per cent where 78 per cent of the population of Kenya lives. On the other hand, there is also a decline in the level of malaria prevalence in endemic areas characterised by a reversal in the age group with the highest prevalence between children less than five years old and those 5-15 years of age. In 2009, a model-based map of the intensity of *P. falciparum* transmission in Kenya as defined by the proportion of infected children aged 2-9 years in the community was produced. Based on the malaria risk map and the eco-epidemiology of malaria in Kenya, districts have been stratified into 4: Lake stable endemic & Coast seasonal stable endemic (risk class equal to or above 20 per cent); Epidemic malaria-prone highland

epidemic-prone districts (risk class 5- <20 per cent); Seasonal low transmission including arid and Semi arid districts (risk class less than 5 per cent); low risk districts (risk class less than 0.1 per cent). Another recent study in Kenya's Nyandarua district that is known to be within the low-risk malaria zones (Njuguna *et al*., 2009) found highest malaria prevalence of 12.2% and 10.2% during July and August, respectively. However, this prevalence was thought to be associated with malaria over-diagnosis, since the same season has a high prevalence of respiratory infections that may have been misdiagnosed as malaria. A case detection study of malaria conducted during the malaria epidemic in 2002 showed a six-fold higher incidence of malaria attacks during the same period, among school children in Nandi epidemic malaria-prone highlands with a low and unstable transmission than in the holoendemic Bondo malaria-endemic lowlands, with intense perennial malaria transmission. The high incidence coupled with the high parasite densities among malaria victims is compatible with a low level of protective immunity in the epidemic malaria-prone highlands. The study concluded that malaria morbidity among school-age children increases as transmission intensity decreases (Clarke *et al*., 2004).

A study conducted in Tanzania found that the prevalence of malaria parasitaemia was lower (11.4%) in children with HIV than in those without HIV; hence HIV infection was negatively correlated with malaria parasitaemia (Eduardo *et al*., 2009). A study conducted in Yemen, by Alserouri *et al*., (2000) on the relationship between asymptomatic parasitaemia and children's cognitive functions was examined in a case control study and then in a natural experiment. Although there was no difference in change in cognitive test scores between those who became non-parasitaemic and those

who remained parasitaemic, the children who initially had the highest parasite density improved the most in two fine motor tests and a picture memory test. The conclusion was that malaria parasitaemia may affect cognition and longer term trial tests were recommended (Al Serouri *et al*., 2000). A review by Trape *et al*., (2002) described a study in Senegal, whose criteria for the diagnosis of *P. falciparum* attacks were based on fever (rectal temperature ≥38°C) and a ratio of parasite to leukocyte higher than the age-dependent pyrogenic threshold observed in this population. Episodes of fever associated with a lower ratio of parasite to leukocyte were also considered as malaria attacks if the peaks of parasitaemia were temporally related to fever occurrence. These malaria attacks with rapid self-control of parasitaemia represented 31.9%, 12.0% and 1.2% of malaria attacks in children 0–5 months, 6–11 months and 12–23 months, respectively.

The diagnosis of malaria is based on microscopic examination of a stained thick and thin blood films or fluorochrome labeling (otherwise known as QBC malaria system test), among others. Antibody detection tests based on immunofluorescence and ELISA technology are also available. Because antibodies appear several days after the onset of symptoms, such tests are appropriate for the emergency setting. The diagnosis of malaria is often difficult in non-immune travellers who have not taken chemoprophylaxis correctly or who have taken presumptive treatment. Microscopic examination remains the primary diagnostic method but can be negative when parasite viability is impaired by chemotherapy or when parasitemia is low. Antigen testing may thus be useful for confirming *P. falciparum* infection (Olivier *et al*., 2000). However, the difficulty of detecting malaria parasites during the microscopic examination of thick and thin blood

smears to diagnose malaria remains a major problem because nearly all peripheral health facilities rely entirely on the method (CDC, 2002).

The need for improved malaria diagnosis is especially important for poorer and more vulnerable populations than the rest; hence an urgent need for improved diagnostic tools that can be used at community and primary-healthcare level (Amexo *et al*., 2004). Under-diagnosis of malarial illness in an endemic area is more commonly the result of failure on the part of the patient to reach a health facility than the result of clinical assessment when at a health facility. In some communities a large majority of those suffering from malarial infection and disease do not come to formal health facilities and are therefore not counted (Greenwood *et al*., 1987). The size of this hidden burden of both uncomplicated and severe malaria is unknown. Under-diagnosis is also a problem in countries with little or no malaria, when either the individual or the health professional may fail to consider the possibility of malaria (Chalumeau *et al*., 2006). Occasionally a non-immune adult may present with a complication (e.g., acute renal failure, severe anemia, coma), and the malarial etiology may not be suspected.

2.6 Anaemia in children with malaria

Anaemia is one of the main complications of malaria among children (Hedberg *et al*, 1993), causing many childhood deaths, especially in Africa (Murphy & Breman, 2001) , although nutritional deficiencies, hookworm infection and HIV all predispose to anaemia in children (Premji *et al*, 1995; Lusingu *et al*., 2004; Ronald *et al*., 2006). Malaria may also cause jaundice, due to haemolysis or breakdown of red blood cells. A study conducted in Tanzania found a significant increase in anaemia prevalence with increase

in malaria parasite density (Kitua *et al*., 1997). Marked prevalence difference of malaria and anaemia may be found in some regions, even among neighbouring communities, as was the case in Ghana (Ronald *et al*., 2006).

Anti-malarial drug resistance exacerbates the situation, by increasing the proportion of children who fail to adequately clear parasitaemia after treatment, and who consequently remain anaemic. Several antimalarial interventions have been shown to prevent anaemia in children, including insecticide-treated nets, residual spraying, malaria chemoprophylaxis and intermittent presumptive treatment of infants. However, insecticide-treated nets decrease all-cause mortality among children aged five and below by approximately 20%; the nets are especially protective against development of anaemia (Crawley, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

3.1 .1 Study Area

Nandi County Study Area

Nandi County (Figure 3.1) is situated in the Western part of the Rift Valley Province and occupies an area of 2839 square kilometers; the district lies between latitudes 0.111^{o} South and 0.561^{o} North and longitudes 34.737^{o} and 35.435^{o} East. It is bordered by the districts of Uasin Gishu, Kericho, Kisumu, Vihiga and Kakamega (Mwanzi, 1977). The clinical and laboratory-based study involving the specific clinical features of malaria among children aged twelve and below was carried out at Kapsabet District Hospital and Kapkangani Health Centre in Nandi County, in the Rift Valley Province, Kenya. Kapkangani location, where the laboratory study was done is within Nandi County in the epidemic malaria-prone highlands of western Kenya, and this is where Kapkangani health centre is situated (about 45km South-East of Kakamega; altitude of about 2000 metres above sea level; latitude, 0.1833333° and longitude, 34.9666667°).

Climate and Economic Activities That Encourage Malaria in Nandi County

The hilly and undulating topographical features of Nandi County coincide with a spatial distribution of ecological zones that define the agricultural and overall economic development potential of the area. The northern parts receive rainfall ranging from 1300mm to 1600mm, while the southern half receives as high as 2000mm per annum; hence the average rainfall ranges from 1200mm to 2000mm per annum (Nandi District Development Plan 1997-2001). Altitude ranges from 1800 to 2500 metres. However, the

high rainfall and the water reservoirs, such as swamps, forest cover and the thickets formed by the trees, tea shrubs and crops as well as river water also tend to favour mosquito breeding, hence resulting in high malaria prevalence.

Kapsabet town is the largest urban centre and is also the district headquarters. Its location along the Eldoret-Chavakali road makes it accessible by tarmac road to the major towns in western Kenya. The other urban centre is Nandi Hills, while other smaller centres include Ndalat, Kipkarren, Kabiyet and Kapkangani.The roads are uniformly distributed throughout the district, except in the hilly areas of Tinderet division in the south eastern region of the County and the forest areas to the west and north western parts of the County (North Nandi and South Nandi Forests). The efficient road network unfortunately also encourages the transmission of malaria because immigrants with malaria from the malaria-endemic lowlands of western Kenya who may be carrying malaria parasites may travel over a short time to the epidemic malaria-prone highland regions, including Nandi and Uasin Gishu Counties. Mosquitoes without malaria may then bite the infected people and in the process transmit malaria parasites from the malaria-endemic lowlands to the epidemic malaria-prone highland population. Kapsabet hospital is one of the major hospitals in Kapsabet town and it is over-utilized, taking into account the fact that together with the other major hospitals, it caters for the whole district, yet its absorption capacity is small.The rural areas of Nandi are served by a number of health centres and dispensaries (Nandi District Development Plan 1997-2001). The rainfall distribution and intensity also has a direct relationship to the economic activities in Nandi County. The areas with 1500mm and above of rainfall per annum form the extended Agro-Ecological Zone for the current and potential tea cultivation. The relatively drier areas are suitable

for maize growing. Dairy activity is practised through out the entire district. Due to the reliability of the rainfall in the entire district Nandi has the potential of producing various agricultural crops ranging from tree crops, horticultural crops, and pyrethrum, cereals and fruit trees (Nandi District Development Plan 1997-2001). Nandi County physiography can be divided into five distinct features; the Kapsabet Figureau, the epidemic malaria-prone highlands and foothills of Tinderet volcanic mass, the Kingwal Swamp in the Baraton-Chepterit area, the rolling hills to the west and the Nyando escarpment at the southern border. The soils in most of the County are deep, well drained and very suitable for crop cultivation thus agricultural activities form the backbone of the economy. Due to the adequate and reliable rainfall, the County has the potential to produce a surplus of diverse agricultural crops, including tree crops, horticultural crops, and pyrethrum, cereals and fruit trees (Kenya Soil Survey, 1986).

Uasin Gishu County Study Area

Uasin Gishu County is one of the counties in Rift Valley Province (RVP), with a total area of 3,327.8Sq.Km^2. It extends between longitude 34° 50′ and 35 ° 37′ east and 0° 03′ and 0° 55′ north (Source: Map data ©2011Google). Four of the health centres, namely, Turbo Health Centre and three other health centres that serve the Moi University community, namely: Chepkoilel, Medical School Health Centre and Main Campus Health based in Kesses that were selected for the study on period prevalence of malaria and other common diseases were done in Uasin Gishu County. Turbo Rural Health Centre, which is situated within the epidemic malaria-prone highlands of western Kenya (about 35km west of Eldoret at an altitude of about 7000 feet (2100 metres) above sea

level; latitude 0° 40' 53" N and longitude 34° 59' 13" E, has had many seasonal outbreaks of malaria (Some, 1994; Akhwale et al., 2004; Shanks et al., 2004). The three health centres that serve the Moi University community are situated in Moi University campuses (Chepkoilel, Moi University main campus and Town campus) near Eldoret town, whose location coordinates are: 0°31′N 35°17′E0.517°N 35.283°E. The district shares common borders with Trans Nzoia District to the north, Marakwet and Keiyo to the east, Koibatek District to the southeast, Kericho District to the south, Nandi to the wets and Lugari District to the North West.

The epidemiological study involving responses from specific cadres of health professionals was carried out in purposively sampled healthcare institutions from the epidemic malaria-prone highland and the malaria-endemic lowland regions of western Kenya. The institutions from the epidemic malaria-prone highlands of western Kenya, where the data was collected were those in the former wider Uasin Gishu district, including: Moi Teaching and Referral Hospital, Moi International Airport health centre, Eldoret Hospital, Eldoret Medi Heal Hospital, Uasin Gishu District Hospital, Family Care Hospital Eldoret, Elgon View Hospital; Ziwa Sub-District hospital, Turbo Health centre, Sosiani Health Centre, Sambut Health Centre; Soy Health Centre; Moi's Bridge Health Centre and Moi's Bridge Catholic Mission health centre, Kesses Health Centre; Moi University health centres in Kesses, Chepkoilel and Town Campus. Those in Nandi districts included: Kapsabet Hospital, Mosoriot Health Centre and Kapkangani Health centre. The institutions from the malaria-endemic lowlands where the data from health professionals were collected were: KEMRI/CDC Kisumu, Walter Reed Kisumu (Kondele), Walter Reed Kombewa, Kombewa District Hospital, Kisumu, Webuye

District Hospital, Bungoma District Hospita, Mumias Mission Hospital, Lugulu Mission Hospital, Kakamega Provincial Hospital, Jaralam Hospital, Star Hospital, Iguhu District Hospital and Mbale District Hospital (Figures 3.2, 3.3 and 3.4).

Figures 3.1, 3.2, 3.3 and 3.4 for this thesis are found in appendices 1, 2, 3 and 4, respectively.

3.1.2 Study Population

The population of Nandi County was estimated at 752, 965 people in 2011, with an annual growth rate of 3.9% (Kenya National Bureau of Statistics: *Population of local authorities*, 2011). Kapsabet Division, which is situated between 0° 12′ North and 35° 06′ East, with a population of 125, 115 people has the highest population density of 276 people per km^2 in an area of 493.7 km^2 in Nandi County (Nandi District Development Plan 2005-2010; Kenya National Bureau of Statistics: Population of local authorities, 2011). The population distribution across the county varies, in some sub-locations population density is 8.61 per km^2 and in others it is as high as 1201.9 per km^2. The population comprise mainly of Nandi people (74%), a sub-group of the Kalenjin who have resided in the area since the 17^{th} or 18^{th} centuries following migration from Southern Sudan via Mount Elgon (Mwanzi, 1977). The population of Kapkangani area consists of indigenous Kalenjin people and numerous Luhya settlers who have moved from the malaria-endemic lowland areas of western Kenya and have purchased land over a period of time (>30 years). The economy is primarily rural subsistence agriculture, with some families growing tea as a cash crop. Other economic opportunities include casual labour on local tea estates. This population is serviced by Kapkangani Government

Health Centre, which has a catchment area of about 20 000 people and a catchment radius of about 20 km (Brooker *et al*., 2004). Kapkangani has been cited in some previous studies that reported about malaria outbreaks in western Kenya highland regions (Chandy *et al*., 1997).Uasin Gishu County, which has a population estimate of 894,179 and whose population density is 267 people per Km^2 is divided into six divisions namely Kapsaret, Ainabkoi, Kesses, Soy, Turbo and Moiben (*Uasin Gishu District Strategic Plan 2005-2010).* The questionnaires for our study were distributed in some of the larger health centres within Uasin Gishu County.

3.2 Research Design

This study was conducted through a quantitative descriptive, comparative survey study design. Quantitative, descriptive research involves collecting data in order to test hypotheses or answer research questions regarding the subjects of the study (Gay *et al*., 2006). A descriptive study is the systematic collection and presentation of data to give a clear picture of a particular situation, people, activities or events. Descriptive survey design was used because it is an efficient way to obtain information needed to describe attitudes and views of the respondents (Cormack, 1982). Survey research design is the systematic gathering of information from a sample population (Benard & Morrison, 1991). Comparative survey research design is mainly used when a researcher intends to examine and describe particular variables in two or more groups; hence to compare the groups. The study design was therefore used to compare the sensitivity of immunochromatographic malaria rapid diagnostic test using whole blood, plasma and serum from children aged twelve and below.

3.3 Sample Population and Sampling Techniques

3.3.1 Target Population

All the children enrolled in the study were from the epidemic malaria-prone highland region of western Kenya, specifically Kapkangani Division of Nandi County. The study involving collection of clinical and laboratory data targeted children aged twelve and below with clinical features of malaria. Children were chosen because they are among the most vulnerable to malaria. The age bracket enabled a wide variety of clinical features to be evaluated against the sensitivity of the immunochromatographic (ICT) malaria rapid diagnostic test.

3.3.2 Sample Population Calculation

The sample size was calculated using the formula cited by Mugenda & Mugenda, (2003) and Kothari (1990) shown below:

$$n = \frac{z^2 pq}{d^2}$$

Where n = the desired sample size

z = the standard normal deviate at the required confidence level.

p = the population in the target population estimation to have characteristics being measured.

q = 1-p

d = the level of statistical significance test.

The average sensitivity for ICT malaria rapid diagnostic test from eleven published researches done in the recent past in malaria-endemic countries is 91.4%; hence ‘p’ in this case was 0.914; ‘q’ was therefore (1-0.914= 0.086) and ‘z’ was 1.96 at 95% confidence level. Based on the published finding that 85% of the 801,000 deaths due to malaria in Sub-Saharan Africa were children aged five and below and the older groups of children therefore accounted for the remaining 15% of the deaths from malaria (WHO, 2008), the proportion of the children aged five and below in the sample population was 113 (85%) while 19 (15%) were children in the sample population, aged five years and above. The minimum child sample size ‘n’ for the clinical and laboratory-based study on sensitivity comparison with ICT malaria rapid test was therefore 121. Based on the same formula by Fishers *et al* the population of health professionals from each of the two regions (i.e. the epidemic malaria-prone highlands and malaria-endemic lowlands, respectively) was 121; hence a total of 242 health professionals. Overall, we studied 556 health professionals (368 from epidemic malaria-prone highlands and 188 from malaria-endemic lowlands, respectively).

3.3.3 Sampling Techniques

The study involving the clinical data comparing the sensitivity of the immunochromatographic malaria rapid diagnostic test with specific clinical features in children aged twelve and below employed systematic sampling technique to select the subjects to be included in the sample. In the systematic sampling technique every K^{th} case in the sample population frame was selected for inclusion in the sample (Mugenda & Mugenda, 2003). Systematic sampling was therefore suitable because the children to be sampled had to queue for treatment and those admitted in hospital were placed on beds

that could be counted in sequence. From the records available at Kapsabet District Hospital, 1393 children were treated between 1st April, 2009 and 31st September, 2009. Assuming that the same trend occurred in 2010, the interval between any two children in the sample population (K) was 1393/121= 11.5. Hence every 12th child with features of malaria was selected for the study until the minimum calculated sample size was obtained. The records at the hospital showed that during the peak rainy season (August-September), when malaria outbreak tends to occur in the study area, the average number of children who were attended for suspected malaria every day was 36; hence an average of three children were selected for the study on daily basis, until the minimum sample size was obtained, between September 2009 and May, 2011.

Purposive sampling was used to determine the healthcare institutions with qualified health professionals. Purposive or judgemental sampling is whereby the investigator wants to study a group or groups with particular characteristics or circumstances (Mugenda & Mugenda, 2003). The groups of interest in this study were the cadre of qualified health professionals trained to handle malaria rapid diagnostic tests or those in another region with the same qualifications; hence the targeted health professionals were professionally eligible to be trained to use malaria rapid diagnostic tests. The qualified health professionals were medical research scientists, consultant medical officers/doctors and clinical officers, nursing officers, public health officers, pharmacists and laboratory technologists/technicians.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

The first inclusion criterion for the study subjects was the presence of malaria.

Definition of malaria: Malaria fever was defined as presence of fever (axillary temperature of at least 37.5 ^{O}C) at the time the blood slide was taken, or a clinical history of fever within the preceding 48 hours in conjunction with a positive blood test for asexual *P. falciparum* malaria parasites at any density on blood slide microscopy or at a density of more than 2500 malaria parasites per microlitre of blood for those aged at least one year (Smith *et al*., 1994). This definition of malaria is preferred because it can be derived by multiple logistic regression and has been found to be more than 80% specific (Smith *et al*., 1994; Mwangi *et al*., 2005). Hence the second inclusion criterion was a child with axillary temperature of at least 37.5 ^{O}C. The third inclusion criterion was informed consent from either the parent or guardian and the fourth criterion was a child aged twelve and below (except neonates).

3.4.2 Exclusion Criteria

The first exclusion criterion was any child aged twelve years and above. The second criterion was any child whose parents or guardians did not give informed consent for the study. Thirdly, neonates were excluded from the study because they have low chances of getting malaria due to possession of maternal antibodies against malaria.

3.5 Data Collection

3.5.1 Research Instruments

A semi-structured questionnaire (shown in appendix 5) was administered by the investigator during each interview schedule as the main tool for data collection. All clinical data collected were subjected to the comparisons of clinical and laboratory examination, respectively, as outlined by the objectives of the study and the research instrument. Semi-structured questionnaires were used to enable the investigator to balance between the quantity and quality of data that was collected. This intricate balance between the quality and quantity of information was useful for a indept explanation of the reliability of malaria rapid diagnostic tests and clinical features of malaria.

3.5.2 Data Collection Procedures

Quantitative types of data were collected from 132 children with features of malaria during the months of September 2009 to May 2011 using structured questionnaires as a guideline for the clinical data collection. The data was collected by the principal investigator, with the help of one research assistant who is professionally trained in clinical examination and treatment of children (a clinical officer) and laboratory technologists based in a CDC/KEMRI-accredited laboratory in Kapsabet District Hospital. Data was collected at the hospital when the children came for malaria treatment. Introductory explanation was given to each parent/guardian of the affected child to enable them give consent for the child to be recruited after accepting and signing the consent agreement form. The questions in the Interview Schedule (appendix 5) were then used as a guideline for the data collection. Physical examination was then conducted on each child, to measure the parameters indicated on the interview schedule

(splenomegaly and hepatomegaly). Each child was then sent to the laboratory for the determination of haemoglobin level and malaria parasitaemia. Briefly, thick and thin blood slides were then prepared and preserved appropriately for malaria parasite quantification.

3.5.2.1 Procedure for the microscopic diagnosis of malaria by thin and thick giemsa staining techniques

Malaria was diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites. The thin films were used for identifying the species of malaria parasites. Briefly, the patient's finger was cleaned with 70% ethyl alcohol, allowed to dry and then the side of fingertip was pricked with a sharp sterile lancet and two drops of blood were placed on a glass slide. To prepare a thick blood film, a blood spot was stirred in a circular motion with the corner of the slide, taking care not to make the preparation too thick, and allowed to dry without the fixative. After drying, the spot was stained with diluted Giemsa (1: 20, vol/vol) for 20 min, and the film placed in buffered water for 3 min. The slide was then allowed to air-dry in a vertical position and examination using a light microscope. As they are unfixed, the red cells lyse when a water-based stain was applied. A thin blood film was prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45° and then smearing the blood with a swift and steady sweep along the surface. The film was then allowed to air-dry and was then fixed with absolute methanol. After drying, the sample was stained with diluted Giemsa (1: 20, vol/vol) for 20 min and the slide was then briefly dipped in and out of a jar of buffered water. The slide was then

allowed to air-dry in a vertical position and examined under a light microscope (Chotivanich *et al*., 2006; Cheesbrough, 1987).

3.5.2.2 Procedure for ICT malaria rapid diagnostic test on whole blood, plasma and serum (CDC, 2007)

Blood sample obtained by venipuncture was then placed into three appropriate containers to enable separation into three samples; one of the blood samples consisted of whole blood while another blood sample was placed into sterile container (a plain cuvette without an anti-coagulant). The third blood sample was placed into a cuvette containing an anti-coagulant. The latter two blood containers were then centrifuged immediately to produce serum and plasma, respectively. In other words, plasma was obtained after centrifuging citrated blood while serum was obtained by centrifuging non-citrated blood. The samples were then each subjected to separate strips of ICT malaria rapid diagnostic tests to determine detection of malaria parasitaemia. Hence the determination of sensitivity and specificity values of Immunochromatographic rapid diagnostic tests using plasma, serum and whole blood were subsequently done, considering microscopy method as the reference gold standard for confirmation of parasitaemia.

3.5.2.2.1 Procedure for ICT malaria rapid diagnostic test on whole blood **(CDC, 2007)**

Five microlitres of blood from the patient, collected using the provided pipette was applied to the sample well of the cassette test. The collected whole blood was used immediately for the test. Reagent A was then added to the sample well, which lyses the whole blood sample and allows migration past the sample pad. After 15 minutes, the presence of specific bands in the test card window indicated whether the patient was

infected with *Plasmodium falciparum* or one of the other 3 species of human malaria. The purple pad contained colloidal gold conjugated antibodies that were directed against HRP2. When a positive sample was applied, *P. falciparum* antigens bind to the gold conjugated antibodies. The antigen/antibody complex continued migration along the test strip where they were captured by immobilized antibodies. When capture occured, a pink-purple control line and a pink-purple test line formed in the window. When a negative sample was applied, only the control line appeared.

3.5.2.2.2 Procedure for ICT malaria rapid diagnostic test on serum (CDC, 2007)

Approximately one milliliter of whole blood was removed from the child and placed in a plain, sterile bottle then centrifuged until the serum was separated from the blood sample after the blood was removed from the patient. Five microlitres of the serum was collected using the provided pipette and applied to the sample well of the cassette test. The serum was then used for the test. Reagent A was then added to the sample well. After 15 minutes, the test cassette was observed for presence of specific bands in the test card window as was done for whole blood.

3.5.2.2.3 Procedure for ICT malaria rapid diagnostic test on plasma (CDC, 2007)

Approximately one milliliter of whole blood was removed from the child patient and placed in a sterile bottle containing EDTA or sequestrene anticoagulant then centriguged until the plasma separates from the whole blood sample. EDTA is used extensively in the analysis of blood. It is an anticoagulant for blood samples for complete blood count. An anticoagulant is a substance that prevents coagulation (clotting) of blood (Lanigan and Yamarik, 2002). Five microlitres of the plasma was collected using the provided pipette

and applied to the sample well of the cassette test. The plasma was then used for the test. Reagent A was then added to the sample well. After 15 minutes, the test cassette was observed for presence of specific bands in the test card window as will be done for whole blood.

3.5.2.3 Procedure for haemoglobin level estimation/measurement

Haemoglobin level estimation was done using the blood haemoglobin photometre (Haemocue® AB, Anglholm, Sweden) developed by Von Schenck *et al*., (1986). A drop of blood was obtained from the sterilized pricked site for this purpose from each patient and placed onto a specifically designed haemoglobin microcuvette, for processing and reading of the results. The WHO definition of anaemia (WHO, 1968) was used during the study, whereby anaemia was defined as Hb below 11g/dL, and categorized as mild (Hb between 8.0-10.9g/dL) and moderate (Hb between 5.1 and 7.9g/dL) anaemia. Severe anaemia was defined as a haemoglobin concentration of <5 g/dl since below this level hospitalized children are often transfused (Lackritz *et al.*, 1992).

3. 5.2.4 Estimation of *Plasmodium Falciparum* Parasite Density

Thick and thin blood smears were prepared, air-dried, and stained using 10% Giemsa for 15 minutes. Parasites and leucocytes were counted in the same field until 200 leucocytes were counted. Asexual *P. falciparum* parasite densities were expressed per µL of blood assuming a mean leucocyte count of 8000 cells/µL (Guerrant *et al*., 1999; Véronique *et al*., 2000).

The detailed procedure for *Plasmodium falciparum* density is outlined as follows: Two drops of blood was obtained from the same pricking site and placed onto a clean blood

slide. One drop was placed on one end of the same slide to make a thin blood film, while a thick blood film was placed on the other end. The thin blood film slides were then fixed using methanol and stained with 10% Giemsa stain and kept in a slide box in readiness for analysis. Thick and thin films were interpreted as negative only after examination with an oil immersion lens at × 1,000 magnification for at least 100 oil immersion fields by an expert microscopist. All the samples were air-dried, fixed in methanol and then stained for 15–30 minutes in Giemsa (BDH Ltd); a 1:10 diluted Giemsa (pH 7.2) were used. The stains were washed off with tap water and the smear examined by × 1,000 magnification. After each slide was examined, it was carefully dipped into xylene to remove the oil emulsion from its surface in readiness for further preservation in a slide box. *Plasmodium* parasite density was calculated using the formula by Guerrant *et al*., (1999) and Véronique *et al*., (2000). The number of parasites per 200 white blood cells (WBC) on the thick blood smear was calculated by multiplying this number by 40 (assuming a white blood cell count of 8000/microlitre of blood). The value obtained was the number of parasites per microlitre of blood. The level of parasitaemia (Kenya; Ministry of Health; 2008**)** was then given, according to the guidelines outlined as mild (<10,000parasites/microlitre of blood), moderate (10, 000-100,000 parasites/microlitre of blood) or high/hyperparasitaemia (>100,000parasites/microlitre of blood in low malaria transmission area or > 200,000parasites/microlitre of blood in high malaria transmission areas).

3.5.2.5 Measurement of the spleen enlargement (splenomegaly) and liver enlargement (hepatomegaly) in children

The measurement of the spleen enlargement (splenomegaly) and liver enlargement (hepatomegaly) was graded according to the standards given by Swill beh, (2002) and Behrman *et al*., (2004).

3.6 Malaria Clinical Features among Children

3.6.1 Malaria clinical features among children aged five years and below

The study used the recommended malaria standard definitions by the World Health Organization (WHO, 2000) for purposes of comparing the clinical features of uncomplicated malaria among children aged five and below, with malaria rapid diagnostic tests. The following clinical features/clinical features were included for children aged five years and below: fever, convulsions associated with fever, headache, backache, joint pains, chills, sweating, myalgia, nausea, vomiting, diarrhea, irritability and refusal to feed; all of these clinical features in the presence of peripheral malaria parasitaemia (Kenya, Ministry of Health, 2008; dcdc@health.go.ke). Measurement of complicated (severe) malaria in children aged five and below included the following clinical features, in addition to peripheral malaria parasitaemia: anaemia (mild, moderate or severe), *some dehydration* characterized by any two or more features evidenced by sunken eyes, sunken fontanelle, restlessness/irritability, thirst and reduced skin turgor; presence of *severe dehydration* characterized by sunken eyes, lethargy or unconsciousness , refusal to feed that was clinically diagnosed or inability to drink fluids and reduced skin turgor; presence of any other known complication of malaria (WHO, 2000; Kenya, Ministry of Health, 2008; dcdc@health.go.ke).

3.6.2 Clinical features of malaria among children aged five years and above

The study used the recommended malaria standard definitions by the World Health Organization, in conjunction with the Kenya's Ministry of Public Health and Sanitation (WHO, 2000; Kenya, Ministry of Health, 2008; dcdc@health.go.ke) for purposes of measuring the clinical features of uncomplicated malaria among children aged above five years, in comparison to the sensitivity of malaria rapid diagnostic tests. The following clinical features/clinical features were included for children aged five years and above: fever, headache, backache, joint pains, chills, sweating, myalgia, nausea, vomiting, all of the clinical features in the presence of malaria parasitaemia. Measurement of complicated malaria in children aged above five years included the following clinical features, in addition to peripheral malaria parasitaemia: anaemia, *some dehydration* characterized by any two or more features evidenced by sunken eyes, sunken fontanelle, restlessness/irritability, thirst and reduced skin turgor; presence of *severe dehydration* characterized by sunken eyes, lethargy or unconsciousness, refusal to feed that will be clinically diagnosed or inability to drink fluids and reduced skin turgor; presence of any other known complication of malaria.

3.7 Statistical Data Analysis

The data collected during the study was analyzed using SPSS (version 16.0, SPSS Inc. Chicago, IL, USA) and by descriptive statistics employing tools of central tendencies, frequency distributions; cross tabulations, Fisher's Exact and Chi-Square (χ^2) of goodness of fit tests, where appropriate. Continuous data were compared by student's t-test and assessed using Pearson's correlation coefficient (R). The percentage frequencies of respondents was used to show the particular frequency of the manifestations of

specific clinical features of malaria, whereby malaria parasitaemia was demonstrated using Giemsa staining microscopy.

CHAPTER FOUR

RESULTS

4.1 Comparison of Immunochromatographic Rapid Diagnostic Test (ICT) Effectiveness using Whole Blood, Plasma and Serum

4.1.1 Specificity of Immunochromatographic Rapid diagnostic test

The results of the study confirmed that immunochromatographic rapid diagnostic test (ICT) is only specific to the diagnosis of *Plasmodium falciparum* and not other species of malaria parasites (Table 4.1). Since 97% of the malaria parasites microscopically identified as *Plasmodium falciparum* tested positive using ICT, the specificity of ICT to *Plasmodium falciparum* was therefore 97%, while the 3% that tested negative using ICT and were microscopically identified as *Plasmodium malariae* (1.5%) and *Plasmodium ovale (1.5%),* respectively (Table 4.1). No child in the sample population had *Plasmodium vivax.* Fisher's exact test showed that there was a highly significant difference in the species occurrence of malaria parasites (Fisher's exact; $p<0.001$) in the sample population.

Table 4.1: Sensitivity of ICT using Plasma, Whole Blood and Serum and Malaria Parasite Species Distribution of Children with Malaria in Western Kenya

Malaria parasite Species found	ICT Test sensitivity (using Whole blood/Plasma)		ICT test sensitivity (using Serum)		**Total (%)**	**Gold Standard (Microscopy) (%)**
	Positive	**Negative**	**Positive**	**Negative**		**Positive**
P. falciparum	114 (86.4%)	14 (10.6%)	112 (84.8%)	16 (12.2%)	**128 (97.0%)**	**128 (97.0%)**
P. malariae	0 (0.0%)	2 (1.5%)	0 (0.0%)	2 (1.5%)	**2 (1.5%)**	**2 (1.5%)**
P. ovale	0 (%)	2 (1.5%)	0 (0.0%)	2 (1.5%)	**2 (1.5%)**	**2 (1.5%)**
P.vivax	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**0 (0.0%)**	**0 (0.0%)**
Total (columns)	**114 (86.4%)**	**18 (13.6%)**	**112 (84.8%)**	**20 (15.2%)**	**132 (100.0%)**	**132 (100.0%)**

Fisher's exact; $p < 0.001$

4.1.2 Sensitivity of Immunochromatographic Rapid diagnostic test

Results in Table 4.1 show that whereas the sensitivity of ICT on plasma and whole blood was the same (86.4%), ICT was less sensitive to malaria diagnosis using serum (84.8%) than whole blood or plasma. However, 89.1%, and 87.5% of the affected children were positive for *P.falciparum* using plasma/whole blood and serum respectively, compared to only 10.9% and 12.5% that were negative for *P.falciparum* using plasma/whole blood and serum, respectively.

4.2 The Training and User-Attitudes of Health Professionals in Western Kenya towards Malaria Diagnostic Tests

Out of the 622 (100%) questionnaires issued to eligible health professionals in the sampled institutions, 556 (89.4%) of the questionnaires were duly filled and returned. Therefore, close to 90% of the eligible target population of health professionals in the health institutions reached during the study responded positively to the questionnaires. Questionnaire respondents from the highlands and lowlands were 368 (66.2%) and 188 (33.8%), respectively. The remaining 66 (10.6%) of the questionnaires were either returned blank or the health professionals to fill them were not on duty or were unreachable due to other reasons. Out of the unavailable questionnaires, 46 (7.4%) and 20 (3.2%) were from the malaria-endemic lowlands and epidemic malaria-prone highlands, respectively.

4.2.1 Responses from Healthcare Personnel about Rapid diagnostic test Training

The responses from healthcare personnel to the questions touching on whether or not they had been trained to use rapid diagnostic tests are shown in Table 4.2.

Across both the epidemic malaria-prone highlands and malaria-endemic lowlands, the results show that 59.2% of the study population in the epidemic malaria-prone highlands and only 22.3% in the malaria-endemic lowlands responded that they had not been trained to use rapid diagnostic tests. Within the epidemic malaria-prone highlands alone, 60.3% of the health professionals responded that they were never trained to use rapid diagnostic tests. This percentage could be higher, given that the percentage of those who did not recommend the use of rapid diagnostic tests on account of lacking the relevant training and experience to use MRDTs was 73.6% (Table 4.6). Within the malaria-

endemic lowlands 76.1% of the health professionals in the malaria-endemic lowlands had been trained to use rapid diagnostic tests, while 23.9% had never been trained to use the same. Pearson Chi-Square test showed a highly significant statistical difference (p <0.001) between those who had been and those who had never been trained to use malaria rapid diagnostic tests (Table 4.2).

Table 4.2: Responses from Healthcare Personnel about MRDT Training Attendance

Question	Response	Region		**Total**
Have you been trained to use malaria MRDT?		**Highlands**	**Lowlands**	
	Yes (%)	146 (26.3%)	143 (25.7%)	**289 (52.0%)**
	No (%)	222 (39.9%)	45 (8.1%)	**267 (48.0%)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0%)**

Pearson Chi Square; Pr <0.001.

4.2. Responses concerning when MRDT Training was done

Across the highland and lowland regions, 51.8% of the health professionals had trained to use rapid diagnostic tests at various times in the past (Table 4.3). Majority (37.8 %) of the health professionals had been trained for the same after the year 2005 while the rest (14.0 %) had been trained before 2005. Less than three percent (2.9%) of all the health professionals in both the epidemic malaria-prone highlands and malaria-endemic lowlands had been trained before the year 2005.

Within the epidemic malaria-prone highlands and malaria-endemic lowlands, majority of the health professionals (28.8% and 55.3% from the epidemic malaria-prone highlands

and malaria-endemic lowlands respectively) who have been trained to use malaria rapid diagnostic tests got their training after the year 2005. Therefore only 9.8% and 22.4% from the epidemic malaria-prone highlands and malaria-endemic lowlands respectively were trained before the year 2005.

The combined data showed that whereas 19.1% and 18.7% of the respondents from the epidemic malaria-prone highlands and malaria-endemic lowlands respectively had been trained within five years prior to our current study, only 6.5% and 7.6% of them had been trained between five to ten years prior to our study. The data (Table 4.3) showed that a much high proportion of the health professionals (61.4% and 22.3% from the epidemic malaria-prone highlands and malaria-endemic lowlands respectively) had never been trained to use rapid diagnostic tests, than those who had been trained; this confirmed the training scenario portrayed by the data in Table 4.2. Pearson Chi-Square showed a highly significant difference ($p < 0.001$) between the rapid diagnostic tests training periods.

Table 4.3: Responses about when MRDT Training was done

Question	Years	Region		**Total**
When were you trained to use malaria MRDT?		**Highlands (%)**	**Lowlands (%)**	
	(<5yrs)	106 (19.1%)	104 (18.7%)	**210 (37.8 %)**
	(5-10yrs)	27 (4.9%)	35 (6.3%)	**62 (11.2 %)**
	(>10yrs)	9 (1.6%)	7 (1.2%)	**16 (2.8 %)**
	Not trained	226 (40.6%)	42 (7.6%)	**268 (48.2 %)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0%)**

Pearson Chi Square; Pr < 0.001.

4.2.3 Types of MRDT the Health Professionals had been trained to use

Whereas the majority (51.3 %) of the health professionals declined to respond about the type of rapid diagnostic test they had been trained to use, 39.2% of health professionals from both the epidemic malaria-prone highlands and malaria-endemic lowlands had been trained to use ParaSight F and Immunochromatographic rapid diagnostic test (ICT) while 9.5% had been trained on the various other rapid diagnostic tests (Table 4.4). Out of those who declined to answer the type of rapid diagnostic tests they had trained to use, majority (65.9%) were from the epidemic malaria-prone highland regions while only 22.8% were from the malaria-endemic lowland regions; hence the data in Table 4.4 confirmed the training scenario in Tables 4.2 and 4.3. Fisher's Exact Test confirmed that there was a

highly significant difference ($p < 0.001$) in the population distribution of health professionals who had trained in various types of malaria rapid diagnostic tests.

Table 4.4: Types of MRDT the Health Professionals had been trained to use

Question	Type of MRDT	Region		Total
Which MRDT were you trained to use?		**Highlands (%)**	**Lowlands (%)**	
	ParaSightF	55 (9.9%)	62 (11.1%)	**117 (21.0 %)**
	ICT	49 (8.8%)	52 (9.4%)	**101 (18.2 %)**
	OptiMAL	4 (0.7%)	15 (2.7%)	**19 (3.4 %)**
	QBC	12 (2.1%)	7 (1.3%)	**19 (3.4 %)**
	Other	6 (1.1%)	9 (1.6%)	**15 (2.7 %)**
	Not trained	242 (43.5%)	43 (7.8%)	**285 (51.3%)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0%)**

Fisher's Exact test; $p < 0.001$.

4.2.4 Attitude Rating towards MRDT Effectiveness in Malaria Diagnosis by the Health Professionals in western Kenya

Across the groups, majority (45.6%) of the health professionals from both the epidemic malaria-prone highlands and the malaria-endemic lowlands responded that malaria rapid diagnostic were effective (2.0%), moderately effective (34.2%) or extremely effective (9.4%) for malaria diagnosis (Table 4.5). However, the rest of the health professionals

(55.4%) declined from responding to the paused question because they had never been trained to use malaria rapid diagnostic tests. The data also showed that majority (84.2 %) of those who did not know about the effectiveness of rapid diagnostic tests were from the epidemic malaria-prone highland regions while the rest (15.8 %) were from the malaria-endemic lowland regions. Hence the data shown in Table 4.5 confirm the training scenario already shown by the data in Tables 4.2 to 4.4. Pearson's Chi-Square showed that there is a highly significant difference (Pearson's Chi Square; Pr < 0.001) in the knowledge distribution about MRDT effectiveness among the health professionals in western Kenya.

Table 4.5: Attitude Rating towards MRDT Effectiveness in Malaria Diagnosis by the Health Professionals in western Kenya

Question	Response	Region		**Total**
How do you rate MRDT effectiveness in malaria diagnosis?		**Highlands (%)**	**Lowlands (%)**	
	Extremely effective	21 (3.8%)	31 (5.6%)	**52 (9.4 %)**
	Moderately effective	86 (15.5%)	104 (18.7%)	**190 (34.2%)**
	Effective	6 (1.1%)	5 (0.9%)	**11 (2.0 %)**
	I don't know/Not trained	255 (45.9%)	48 (8.6%)	**303 (54.4 %)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0 %)**

Pearson Chi Square; Pr < 0.001.

4.2.5 Health Professionals' Responses about MRDT Recommended for wider use with microscopy

Table 4.6 shows the proportion of responses from health professionals on their recommendations on use of rapid diagnostic tests for malaria diagnosis. Across the groups, 59% of the health professionals from both the epidemic malaria-prone highlands and malaria-endemic lowlands declined to recommend any of the rapid diagnostic tests for malaria diagnosis because they had never been trained to use malaria rapid diagnostic tests; 15.1% and 11.2% of them recommended ICT and ParaSight F, respectively, while 10.7% recommended the other types of rapid diagnostic tests. A further 1.3 % of the health professionals recommended all the rapid tests but 2.7% recommended none for wider use. However, 4.1 % of the health professionals recommended other rapid diagnostic tests not shown among the choices provided (most of them specified Parachek rapid diagnostic test). It was noted that 73.6 % of the health professionals from the epidemic malaria-prone highlands declined to recommend any of the rapid diagnostic tests for malaria diagnosis; hence only 26.4 % recommended. The main reason for their decline to respond to the question was their lack of training or exposure to the use of rapid diagnostic tests. However, unlike in the epidemic malaria-prone highlands, the proportion of health professionals from the malaria-endemic lowlands who declined to recommend the use of malaria rapid diagnostic tests was only 30.3 %. Hence 69.7 % of the health professionals from the malaria-endemic lowlands had been trained or had already had an experience of using rapid diagnostic tests for malaria diagnosis. The data therefore confirmed the findings shown by the data presented in Tables 4.2 to 4.5. Fisher's exact test showed a highly significant difference (Fisher's Exact Test; $p<0.0001$)

among those who recommended and those who did not recommend the use of rapid diagnostic tests for malaria diagnosis.

Table 4.6: Health Professionals' Responses about MRDT for wider use with microscopy

Question	Type of MRDT	Region		**Total**
Which MRDT would you recommend for wider use with microscopy?		Highlands (%)	Lowlands (%)	
	Parasightf	25 (4.5%)	37 (6.7%)	**62 (11.2 %)**
	ICT	37 (6.7%)	47 (8.4%)	**84 (15.1 %)**
	OptiMAL	4 (0.7%)	9 (1.6%)	**13 (2.3%)**
	QBC	15 (2.7%)	9 (1.6%)	**24 (4.3 %)**
	Other-specified	0 (0.0 %)	23 (4.1%)	**23 (4.1 %)**
	None	10 (1.8%)	5 (0.9%)	**15 (2.7 %)**
	All	6 (1.1%)	1 (0.2%)	**7 (1.3 %)**
	No MRDT training/experience	271 (48.7%)	57 (10.3%)	**328 (59.0 %)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0 %)**

Fisher's Exact Test; $p<0.0001$.

4.2.6 Health Professionals' Personal Opinions on MRDT use

The data presented in Table 4.7 show that whereas 52.7 % of the combined population of health professionals from both the epidemic malaria-prone highlands and malaria-endemic lowlands recommended rapid diagnostic tests in addition to microscopic examination, 13.8% did not recommend while 33.5% were not sure whether or not to recommend the tests for malaria diagnosis. A comparison of responses within the regions

showed that 49.2% of the health professionals from the epidemic malaria-prone highlands and 59.6 % from the malaria-endemic lowlands recommended the rapid diagnostic tests. However, only 3.7 % of the health professionals from the malaria-endemic lowlands compared with 19.0% from the epidemic malaria-prone highlands did not recommend the rapid diagnostic tests for malaria diagnosis. Across the highland and lowland regions, 12.6% and only 1.2% of the health professionals respectively did not recommend rapid diagnostic tests for malaria diagnosis (Table 4.7). Pearson Chi-Square showed a highly significant difference (Pr <0.0001) between the health professionals who recommended and those who did not recommend the use of malaria rapid diagnostic tests for malaria diagnosis.

Table 4.7: Health professionals' opinions on MRDT use

Question	Response	Region		**Total**
Based on your own opinion, would you recommend the use of MRDT for malaria diagnosis?		**Highlands (%)**	**Lowlands (%)**	
	Yes	181 (32.6%)	112 (20.1%)	**293 (52.7 %)**
	No	70 (12.6%)	7 (1.2%)	**77 (13.8 %)**
	No Comment	117 (21.1%)	69 (12.4%)	**186 (33.5 %)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0 %)**

Pearson Chi Square; Pr < 0.0001.

4.2.7 Responses about the Availability of MRDT supplies in Various Health Institutions

The proportions of health professionals from the epidemic malaria-prone highlands and malaria-endemic lowlands respectively, which responded positively to the question whether or not they had rapid diagnostic tests in their health institutions, were 27.2 % and 52.1 % (Table 4.8). However, the respective proportions from the epidemic malaria-prone highlands and malaria-endemic lowlands that responded negatively to the question whether or not they had rapid diagnostic tests in their institutions were 39.9 % and 19.7%. The data presented in Table 4.8 showed that the proportion of health professionals from the epidemic malaria-prone highlands and malaria-endemic lowlands who didn't know whether or not they had rapid diagnostic tests in their institutions were 32.9 % and 28.2 %, respectively.

Table 4.8: Responses about Availability of MRDT supplies in various Health Institutions

Question	Response	Region		**Total**
Are malaria rapid diagnostic tests currently available in your health institution?		**Highlands (%)**	**Lowlands (%)**	
	Yes	100 (18.0%)	98 (17.6%)	**198 (35.6 %)**
	No	147 (26.3%)	37 (6.7%)	**184 (33.0 %)**
	I don't know	121 (21.9%)	53 (9.5%)	**174 (31.4%)**
	Total	**368 (66.2%)**	**188 (33.8%)**	**556 (100.0 %)**

4.2.8 Cadre Distribution of Healthcare Respondents with Respect to Gender and Regions

The cadre distribution of health professionals who participated in the study and their corresponding percentage proportions are shown in Table 4.9. Overall, approximately 60% (8.6% senior and 51.1% middle-level) of the workers were medical prescribers (medical doctors, pharmacists, clinical officers or nurses), while the remaining 41.3% were laboratory personnel (technicians or technologists). The senior cadre health professionals had the lowest percentage cadre distribution of respondents, consisting of 2.0% and 6.6 % in the epidemic malaria-prone highland and malaria-endemic lowland regions, respectively. However, a relatively higher proportion (19.7%) of senior cadre health professionals participated in the malaria-endemic lowlands than in the epidemic malaria-prone highlands with only 3.0%. Majority (43.9 %) of the health professionals from the epidemic malaria-prone highland region belonged to the middle-level clinical (prescribers) cadre, while about half that proportion (22.3 %) belonged to the middle-level laboratory healthcare cadre. The highest proportion (18.0 %) of the health professionals from the malaria-endemic lowland region comprised of the middle-level clinical cadres whose percentage was 15.8%. Whereas (91.4 %) were from the middle level clinical and laboratory cadres, only 8.6 % of the respondents were from the senior cadre of health professionals (Table 4.9). Pearson's Chi-Square test showed a highly significant statistical difference (Pearson's Chi-Square; $Pr < 0.0001$) among the cadre distribution of health professionals in the epidemic malaria-prone highland and malaria-endemic lowland regions, who participated in the study.

Table 4.9: Cadre Distribution of Healthcare Respondents with respect to Regions

Region	Cadre Distribution			**Total**
	Senior (%)	**Clinical Middle Level (%)**	**Laboratory Middle Level (%)**	
Highlands	11 (2.0%)	233 (41.9%)	124 (22.3%)	**368 (66.2%)**
Lowlands	37 (6.6%)	51 (9.1%)	100 (18.0%)	**188 (33.8%)**
Total	**48 (8.6 %)**	**284 (51.1 %)**	**224 (40.3 %)**	**556 (100.0%)**

Pearson's Chi-Square; Pr < 0.0001

4.3 Predominant Malaria Clinical Features among Children (<12 yrs)

4.3.1 Predominant Malaria Clinical Features across Age groups of the affected children

Based on the sensitivity of immunochromatographic rapid diagnostic test and microscopic examination of malaria parasites, a summary of the commonest clinical features of malaria in descending order of their frequency and the age distribution of the affected children as well as their statistical significance (p values) are shown in table 4.10. Fever was the main symptom that affected 92.4% of the child population with malaria. However, 81.1% of the children with malaria had sweating that was associated fever, with 28.0% of the children presenting also with convulsions and 56.0% of the children having been given fever-reducing drugs (anti-pyretics) or physical tepid sponging to reduce the fever while still at home. The proportions of children with chills and vomiting were 69.7% and 67.4% respectively. Nausea, headache, joint pains, muscle

pains and backache were reported by older children (aged five and above). Nausea that preceded vomiting was reported by 47% of the children. Headache was reported among 65.2% of the children although this was a complaint among older children who could verbally report on it. The proportion of children with diarrhoea was 43.9% and this symptom was mainly among children aged five and below. Myalgia (muscle aches) and backache each presented among 19.7% of the children, while joint pains occurred among 15.9% of the children with malaria. The proportion of children who had already been given anti-malarial drugs at home prior to hospital treatment was 8.3% (Table 4.10).

Table 4.10 is presented in appendix 5 of this thesis.

4.3.2 Anaemia distribution of the children Studied (<12 years)

Laboratory examination of blood revealed anaemia among 85.6 % of the children with malaria across the age groups, whereby 34.1%, 32.6% and 17.4% were aged 0-60months, 61-12months and 121-144months respectively (Table 4.10). Across the age groups of the children with anaemia in the sample population, 54.6 %, 23.5% and 7.6% of them had moderate anaemia, severe and mild anaemia, respectively. Therefore, only 14.4% of the children with malaria had no associated anaemia (Table 4.11). There was a significant difference between the presence and absence of anaemia among the children in the sample population (Fisher's exact Test = 0.002). Within the age groups of the children in the sample population, majority of the children aged 0-5 years had moderate (51%) and severe (26.4%) anaemia, while only 7.5% had severe anaemia. Similarly, out of the children aged 5-10 years, majority of them had moderate (65.3%) and severe (20.4%) anaemia, while only 4.1% had mild anaemia. Out of the children aged 121-144months

(10-12 years) with anaemia, 43.3%, 23.3% and 13.3% had moderate, severe and mild anaemia, respectively). Fisher's exact test showed a significant difference in anaemia severity distribution within the age groups of affected children (Fisher's exact test; p = 0.001).

Table 4.11: Anaemia Severity Distribution among children (<12 yrs) with malaria; (n=132).

Haemoglobin Level (g/dl)	0-60months (%); n=53	61-120months (%); n=49	121-144mon (%); n=30	**Total (%); n=132**
Normal Hb level (>11.0g/dl)	8 (6.1%)	5 (3.8%)	6 (4.5%)	**19 (14.4%)**
Mild Anaemia (8-10.9 g/dl)	4 (3.0%)	2 (1.5%)	4 (3.0%)	**10 (7.6%)**
Moderate Anaemia(5.0-7.9 g/dl)	27 (20.5%)	32 (24.2%)	13 (9.8%)	**72 (54.5%)**
Severe (<5.0 g/dl)	14 (10.6%)	10 (7.6%)	7 (5.3%)	**31 (23.5%)**
Total	**53 (40.2%)**	**49 (37.1%)**	**30 (22.7%)**	**132 (100.0%)**

Fisher's exact Test = 0.002.

4.3.3 Malaria Parasite Density of Children (<12 years) studied

The age distribution of children and their respective parasite densities are shown in Table 4.12. Across the age groups of the children in the sample population, 47.7% of them had moderate malaria parasite density, while 31.8%, 15.2% and 5.3% had mild, high and very high malaria parasite densities, respectively. Children aged five or older had lower proportions of mild (6.1%), moderate (12.1%), high (2.3%) and very high (2.3%) malaria parasitaemia than the younger (< 5 years) children. Fisher's exact test showed a

significant difference in malaria parasite density across age groups of the affected children (Fisher's Exact Test; $p < 0.002$).

Within age groups of the children with malaria, 51.0%, 32.1%, 15.1% and 1.9% of the children aged 0-5 years had moderate, mild, high and very high malaria parasite densities, respectively. Similarly, 40.8%, 34.7%, 18.4% and 6.1% of the children aged 5-10 years had moderate, mild, high and very high malaria parasite densities, respectively. Within those aged 10-12 years, 53.3%, 26.7%, 10.0% and 10.0% had moderate, mild, high and very high malaria parasite densities, respectively. There was no significant difference (Fisher's exact test; $p< 0.002$) in the distribution of malaria parasites within the age groups and across the age groups of the affected children.

Table 4.12: Malaria Parasite Densities of affected children (<12 yrs) studied

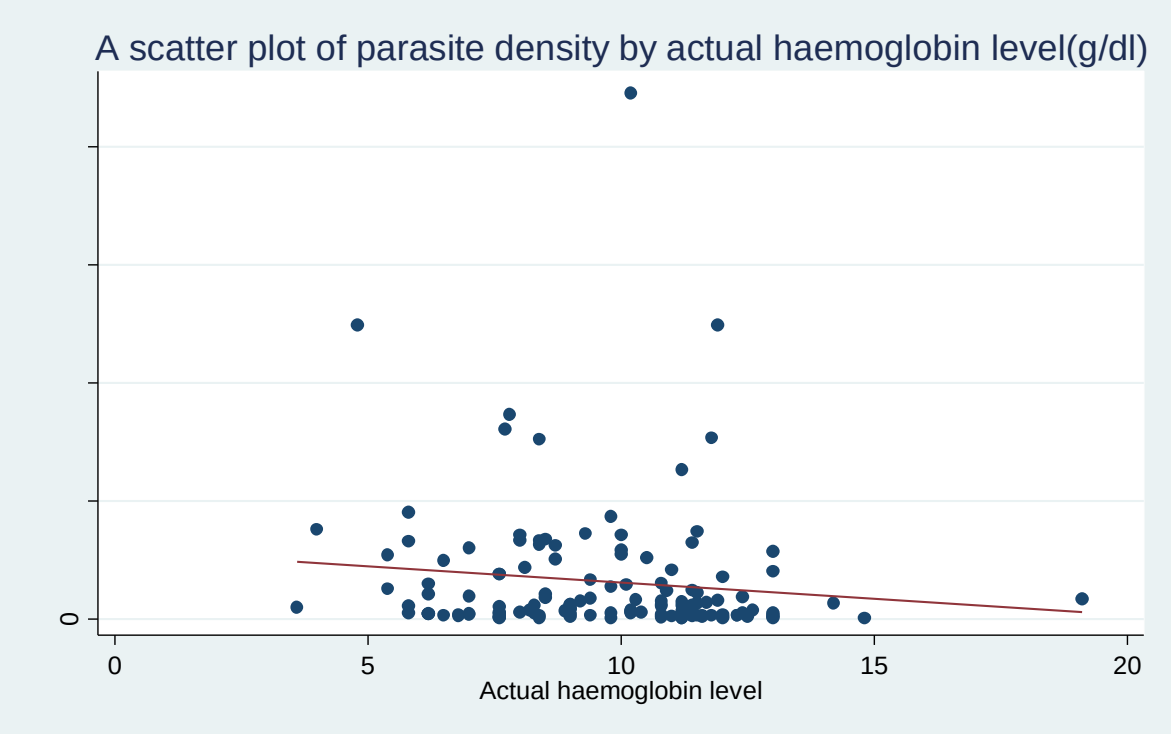
Malaria Parasite Density (per µL of blood)	0-60 months (%)	61-120 Months (%)	121-144 Months (%)	**Total (%)**
Mild (<10,000/µL)	17 (12.9%)	17 (12.9%)	8 (6.1%)	**42 (31.8%)**
Moderate (10,000-100,000/ µL)	27 (20.5%)	20 (15.2%)	16 (12.1%)	**63 (47.7%)**
High (100,000-200, 000/ µL)	8 (6.1%)	9 (6.8%)	3 (2.3%)	**20 (15.2%)**
Very high (>200,000/ µL)	1 (0.8%)	3 (2.3%)	3 (2.3%)	**7 (5.3%)**
Total (%)	**53 (40.2%)**	**49 (37.1%)**	**30 (22.7%)**	**132 (100.0%)**

Fisher's Exact Test; $p< 0.002$.

4.4 Relationship between Malaria Parasite Density and Predominantly elicited Clinical Features of Malaria among affected Children

4.4.1 Correlation of Malaria Parasite Density and Haemoglobin Levels

Malaria parasite density for the affected children ranged between 307 and 891423, with a mean of 63607 malaria parasites per microlitre of blood. Pearson's analysis showed a negative correlation between haemoglobin levels and malaria parasite density, such that as the parasite density increased, the haemoglobin level reduced ('R' =-0.1171; p =0.181). However, the negative correlation between the actual haemoglobin levels of the children in the sample population and their malaria parasite densities was statistically insignificant. The scatter plot of the statistical data for the correlation of actual haemoglobin levels and the malaria parasite densities for all the children in the sample population (n= 132) are shown in Figure 4.1.



p = 0.181

Figure 4.1: Correlation of Actual Haemoglobin levels with Malaria Parasite Densities among the children studied.

4.4.2 Relationship between Malaria Parasite Density and Anaemia

Out of the 85.6% of children in the sample population with malaria-associated anaemia, 28.2%, 47.7%, 17.1% and 6.3% of them had mild, moderated, high or very high malaria parasitaemia, respectively. However, 15.9% of the children with malaria in the sample population had no anaemia but had mild (47.6%), moderate (47.6%) or high (4.8%) malaria parasite density. There was a highly significant difference in malaria parasite density distribution among the studied children with anaemia (Fisher's Exact Test; $p < 0.0001$).

Across the various levels of malaria-associated anaemia, 7.1%, 42.9%, 26.2% and 23.8% of the affected children had mild malaria parasite density. Among those with moderate malaria parasite density, 6.3%, 61.9%, 19.0% and 12.7% of the affected children had various levels of anaemia. Relatively fewer children (20.5%) in the sample population with various grades of anaemia had high (15.2%) or very high (5.3%) malaria parasitaemia (Table 4.13).

Table 4.13: Distribution of Malaria Parasitaemia levels and Anaemia Severity of the children studied

Malaria Parasite Density (per µL of blood)	Severity of Anaemia				**Total**
	Mild Anaemia (11.0-11.9g/dl)	Moderate anaemia (8.0-10.9g/dl)	Severe anaemia (<8.0g/dl)	No anaemia (>12.0g/dl)	
Mild (<10,000/uL)	3 (2.3%)	18 (13.6%)	11 (8.3%)	10 (7.6%)	**42 (31.8%)**
Moderate (10,000-100,000/uL)	4 (3.0%)	39 (29.5%)	12 (9.1%)	8 (6.1%)	**63 (47.7%)**
High (100,000-200,000/uL)	2 (1.5%)	12 (9.1%)	5 (3.8%)	1 (0.8%)	**20 (15.2%)**
Hyperparasitaemia (Very high [>200,000/uL])	1 (0.8%)	3 (2.3%)	3 (2.3%)	0 (0.0%)	**7 (100.0%)**
Total	**10 (7.6%)**	**72 (54.5%)**	**31 (23.5%)**	**19 (14.4%)**	**132 (100.0%)**

Within the various levels of malaria-associated anaemia, 30.0%, 40.0%, 20.0% and 10.0% of the children with mild anaemia had mild, moderate, high or very high malaria parasitaemia, respectively. Among the children with moderate anaemia, 25.0%, 54.2%, 16.7% and 4.2% had mild, moderate, high or very high malaria parasitaemia. However, among those with severe anaemia, 35.5%, 38.7%, 16.1% and 9.7% had mild, moderate, high or very high malaria parasitaemia, while among those without anaemia, 52.6%, 42.1%, and 5.3% had mild, moderate and high malaria parasitaemia. However, no child without anaemia had very high malaria parasitaemia. There was no significant difference

in the distribution of malaria parasite density within or across various grades of anaemia among the affected children (Table 4.13).

4.4.3 Relationship between Malaria Parasite Density and Jaundice

Across the child population with malaria-associated jaundice, 21.4%, 20.6% and 35.0% had mild, moderate, high and very high malaria parasitaemia, respectively. Within the child population with malaria-associated jaundice, 31.0%, 44.8% and 24.1% had mild, moderate and high malaria parasitaemia, respectively while none had very high malaria parasitaemia.

However, Pearson's Chi-Square showed no significant relationship between jaundice and malaria parasite densities across and within the affected child population (Pearson's Chi-Square; $p>0.05$).

4.4.4 Relationship between Malaria Parasite Density, Hepatomegaly and Splenomegaly

Among the 11.4% proportion of children with hepatomegaly the proportion with mild, moderate, high and very high malaria parasite density in the sample population constituted 4.5%, 3.8%, 1.5%, and 1.5% respectively. Fisher's Exact test showed no significant relationship ($p>0.05$) between malaria parasite density and hepatomegaly among the children in the sample population. Fisher's exact test also showed no significant relationship ($p>0.05$) between malaria parasite density and splenomegaly. Splenomegaly was most frequently associated with moderate to severe anaemia among the children with malaria. However, Fisher's exact test showed no significant relationship ($p>0.05$) between splenomegaly and actual haemoglobin levels.

4.5 Malaria Diagnosis Outcomes from selected Rural health centres within the Epidemic Malaria-Prone Highland Region of Western Kenya

4.5.1 Prominence of Malaria Prevalence in selected Rural health centres in the Epidemic malaria-prone highlands of western Kenya

Malaria remains among the most prevalent diseases that affect the human population living within the epidemic malaria-prone highlands of western Kenya. Tables 4.14, 4.15, 4.16, 4.17 and 4.18 show the prevalence of the five commonest diseases diagnosed, especially among children during the period between 2005 and 2010, in the selected rural health centres within the epidemic malaria-prone highland region of western Kenya. The results show that malaria occupies either the first or second place among the top five diseases as far as prevalence is concerned (Figures 4.2, 4.3 and Tables 4.15 to 4.18). The period prevalence of malaria in two health centres situated within the epidemic malaria-prone highlands of western Kenya where past published researches reported malaria epidemics, has consistently remained higher than all other common diseases in that region, within the last five years (2005-2010). Turbo Health Centre that is situated in Uasin Gishu County in Rift Valley Province in Kenya has recorded malaria period prevalence of between 32.1% in 2005 and 48.8% in 2010; hence an average malaria period prevalence of 41.8%. However, the average period prevalence of the second most prevalent disease in the same health centre over the same period (2005-2010) was 19.0%, while other common diseases had even much lower period prevalence (Table 4.17). Similarly, the period prevalence of malaria in another health centre (Kapkangani) situated in the neighbouring Nandi County also within the Kenya epidemic malaria-prone highlands has recorded an average malaria period prevalence of 29.6% during the period between 2005 and 2010. The period prevalence of malaria at Kapkangani ranged between

21.4% in 2005 and 31.3% in 2010. However, the average period prevalence of the second most prevalent disease (upper respiratory tract infection) in the region during the same period was 25.2%, while other common diseases had even much lower period prevalence (Table 4.18). Results from each of the three other health centres situated within a higher and comparatively colder altitude where Moi University Campuses around Eldoret (Chepkoilel, Town campus and Main Kesses campus health centres respectively) are found also showed that the period prevalence of malaria was second, after upper respiratory infections. The period prevalence of the commonest disease in the Main Kesses Campus of Moi University (upper respitatory infections) from 2007 to 2010 was: 26.0%, 29.7%, 25.3% and 28.9%; hence an average period prevalence of 27.5%. However, the period prevalence of malaria in the same health centre within the same period was: 19.4%, 27.7%, 31.0% and 21.8%; hence an average malaria period prevalence of 25.0% (Table 4.17). The period prevalence of the commonest disease in Town (Medical School) Campus Health centre of Moi University (upper respitatory infections) from 2007 to 2010 was: 29.3%, 21.2 %, 29.9% and 25.5 %; hence an average period prevalence of 26.5%. However, the period prevalence of malaria in the same health centre within the same period was: 12.5%, 14.7%, 12.4% and 16.4%; hence an average malaria period prevalence of 14.0 % (Table 4.18).The period prevalence of the commonest disease in Chepkoilel Campus Health centre of Moi University (upper respitatory infections) from 2007 to 2010 was: 37.7%, 43.5%, 25.6% and 27.1%; hence an average period prevalence of 33.5%. However, the period prevalence of malaria in the same health centre within the same period was: 36.9%, 41.2 %, 10.1% and 23.2 %; hence an average malaria period prevalence of 27.9%.

Tables 4.14, 4.15, 4.16, 4.17, 4.18 and 4.19 and Figures 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10 are presented in the appendices pages of this thesis.

4.5.2 Analysis of Malaria Diagnosis across Rural health centres in the highlands of western Kenya

A retrospective study of malaria diagnosis and its challenges across five rural health centres (Turbo, Medical School and Chepkoilel University College health centres) all situated within the epidemic malaria-prone highlands of western Kenya was done during the year 2007. The results are presented in Table 4.19. Out of the total 153,530 patients evaluated for possible malaria across five highland-based health centres during 2001-2010 periods, 58.3% were presumptively treated, 33.3% had no malaria parasites and 8.4% had malaria parasites. The presumptively treated patients were those who were recorded as having been treated for malaria without laboratory confirmation of malaria or regardless of absence of malaria parasites from laboratory results.

4.5.2.1 Analysis across three Rural health centres (Turbo, Medical School and Chepkoilel) during the year 2007

Results for the analysis across the three rural health centres (Turbo, Medical School and Chepkoilel) during the year 2007) are shown in Figures 4.2 and 4.3. Paired sample T- test showed a highly significant difference between the presumptively treated and laboratory-confirmed malaria across three (n=9771; mean=1.132E3; 95% C.I: 918.517 - 1.346E3; $p < 0.001$) and across two (n=118,255; mean=8119.571; 95% C.I: 5836.924-10402.219; $p <0.001$) highland-based health centres in western Kenya during years 2007 and 2001-2007, respectively.

Another general finding about malaria diagnosis across and within various health centres was that the proportion of presumptively treated patients was significantly higher than those microscopically confirmed to have malaria parasitaemia. The proportion of those who had suspected but found not to have malaria (MPS negative) was also significantly higher than those with confirmed malaria. Figure 4.3 also shows one peak malaria outbreak season between January and April and another peak around and July of the year 2007, which was followed by a gradual reduction of malaria to the lowest level towards the end of that year.

4.5.2.2 Analysis of malaria diagnosis across two Rural health centres (Chepkoilel and Turbo) during the period between 2001 and 2007

During the period between 2001 and 2007, the total number of patients evaluated for possible malaria diagnosis in the two rural health centres (Figures 4.4 and 4.5) were 118,255(77.02%) out of the overall total of 153, 530 (100%) evaluated in all the five rural health centres during the period. Out of the total evaluated the number that tested negative for malaria was 34,246 (22.3%) while those confirmed to actually have malaria were 10,358 (6.7%). However, the proportion that was presumptively treated for malaria was 73,651(48.0%). A paired sample T- test was also carried out to test the equality of means between the total number of patients presumptively treated and the total number confirmed to have malaria in the two health centres for the years 2001-2007 showed a highly significant statistical difference ($n = 118,255$; mean=8119.571; standard error=932.869; 95% confidence interval of the differences: 5836.924-10402.219; $p < 0.001$). However, there was no statistical significance between the patients treated for malaria and those confirmed to have malaria in the two health centres ($p = 0.415$).

A notable general observation in the diagnosis of malaria during the period between 2001 and 2007 was the general reduction in the total number of patients evaluated for possible malaria. This was observed across the selected health centres (Figures 4.6) and within the various health centres in the epidemic malaria-prone highland region (Figures 4.6 to 4.10).

CHAPTER FIVE

DISCUSSION

The results from the current study confirmed that immunochromatographic rapid diagnostic test (ICT) is only sensitive to *Plasmodium falciparum* and not other species of malaria parasites. Although there was no significant difference in the sensitivity of ICT on plasma, whole blood or serum, the results suggested that ICT was less sensitive to serum compared to plasma or whole blood. Hence serum should be less relied upon than whole blood or plasma for the detection of malaria.

The differences in the sensitivity in the detection of malaria-associated antigens in the blood of the affected children enrolled for the current study occurred possibly because there were more circulating malaria-associated antigens in whole blood/and plasma than in serum. The application of whole blood specimen onto the prepared rapid diagnostic strip was done almost immediately following blood collection because no other procedure was required before testing the blood for circulating antigens. This meant that the blood sample used for detecting antigens in whole blood had not evem clotted in the process. However, the same results were obtained from the blood sample that had been subjected to an anticoagulant required for the preparation of plasma after centrifuging the blood. Both the whole blood and the plasma therefore possibly had higher concentrations of malaria-associated antigens that had not been adversely affected by blood clotting. If this was the case, then it implies that blood clotting renders some malaria-associated antigens inaccessible for the immobilized antibody in the rapid diagnostic test, unlike in the case of serum, whose preparation involved centrifuging of blood that had no anticoagulant. Hence blood coagulation (as in serum preparation) may have reduced

circulating antigens, making their detection less effective than in plasma (with anticoagulant). More studies need to be carried out to establish the factors responsible for the differences in the sensitivity of whole blood, plasma and serum towards the immobilized antibody in the rapid diagnostic test strips.

Prior to the current study, scanty data about the test performance of rapid diagnostic tests on children as regards the study of febrile illnesses (Gasser *et al*., 2005) were available. There was therefore a need to resolve the issue that concerns the diagnostic testing of young children with clinical illnesses resembling malaria in regions where *Plasmodium falciparum* greatly predominates (Murray *et al*., 2008). Furthermore, among the unresolved issues in past studies was whether or not the rapid diagnostic tests were equally sensitive when used on plasma, serum or whole blood (Gasser *et al*., 2005; Murray *et al*., 2008).

The predominance of *Plasmodium falciparum* in the current study was consistent with findings from one previous study (Arness *et al*., 2003) conducted within the same epidemic malaria-prone highland region, whereby *Plasmodium falciparum* distribution was >99%. The results of the current study are also consistent with the national statistics in Kenya whereby *Plasmodium falciparum* was found to be the predominant species (98.2%) while *Plasmodium malariae* is 1.8%. However, *Plasmodium vivax* accounted for up to 40-50 per cent of infections in the Northern and North Eastern parts of Kenya where the prevalence of malaria is on average <0.1 per cent (Division of Malaria Control, Kenya, 2011). Hence the introduction of new methods that detect malaria regardless of absence or presence of supportive clinical features, such as the immunochromatographic

rapid diagnostic test that was used in the current study is indeed an important milestone in the diagnosis of malaria. Furthermore, other previous researches have reported that *Plasmodium falciparum* malaria is extensive, prevalent, and increasing in sub-Saharan Africa (Snow, *et al*., 1997; Craig *et al*., 1999; Snow *et al*., 2001); hence a major reason for our emphasis on introduction of improved malaria diagnostic techniques.

Malaria diagnosis is increasingly receiving much attention due to the observed widespread high rate of malaria misdiagnosis (Reyburn *et al*., 2004) and adoption of more expensive anti-malarial drugs. Although the widespread implementation of malaria control interventions such as insecticide-treated bed nets (ITNs) and artemisinin combination therapy (ACT) have resulted in dramatic reductions of transmission in areas where malaria is endemic (Lindblade *et al*., 2004; Bhattarai *et al.*, 2007; Okiro *et al*., 2007), malaria diagnosis remains a challenge in the highland areas (>1,500 m above sea level) where unstable malaria transmission that causes low malaria incidence during dry seasons (Ernst *et al*., 2006) commonly occurs. A case in point was the results of one recent study in Kipsamoite and Kapsisiywa sites (Chandy *et al*., 2009), a few kilometers from Kapkangani where we conducted our study. Chandy *et al* (2009) found that a large number of patients with symptoms suggestive of malaria were either found to have no *Plasmodium falciparum* malaria parasites or had low gametocyte prevalence according to microscopy method, during the low transmission seasons for the period between 2003 and 2008. In some instances during the same study, malaria parasitaemia was only detected by use of PCR method. Yet another challenge encountered during the study by Chandy *et al* (2009) was the presence of asexual parasitaemia and gametocytemia in asymptomatic persons; such a situation is potentially dangerous in the highland region

because the lack of malaria symptoms in a patient with malaria parasitaemia may raise least suspicion of malaria (consequently leading to delayed or no malaria treatment) from the attending clinicians. Furthermore, during the same study (Chandy *et al*., 2009) the daily temperatures and rainfall patterns did not differ significantly during the years with high or low incidences of malaria. It is because of such diagnostic challenges that the existing malaria diagnostic methods should either be improved or supported by other additional cost-effective diagnostic methods.

The introduction of malaria rapid diagnostic tests (Jelinek *et al*., 1998; Makler *et al*., 1998; Premji *et al*., 1999; Jelinek *et al*., 1999; Jamshaid *et al*., 2003; Mens *et al*., 2006; Hawkes & Kain, 2007; Mendoza *et al*., 2007; Mlambo *et al*., 2008; Mens *et al*., 2008) and other alternative methods of malaria diagnosis, such as Polymerase chain reaction (Snounou *et al*., 1993; Tan *et al*., 1997; Morassin *et al*., 2002; Hamer *et al.*, 2002; Swan *et al*., 2005; Murray *et al*., 2008) and others used over the years (Reinhartz *et al*., 1993; Scholl *et al*., 2004; Padial *et al*., 2005; Poon *et al*., 2006; Oh *et al*., 2007; She *et al*., 2007; Park, 2008; Patarakul, 2008) have triggered much interest among scientists who are researching on malaria. However, majority of published studies have evaluated malaria rapid diagnostic tests (MRDT) in health care settings where trained personnel performed the assay and microscopy. Although the malaria rapid diagnostic tests perform optimally in the targeted patient population with febrile illness from such health care settings, there are far fewer data on test performance for children (Gasser et al., 2005) and much less so in peripheral health facilities situated in more rural setups and that typically serve a higher population than the urbanized hospitals with more medical expertise.

The results of the current study also showed that there was no significant relationship between jaundice and malaria parasite densities across and within the affected child population. Malaria parasites may cause red blood cell destruction (by the process of haemolysis). If haemolysis persists, jaundice may occur due to accumulation of bilirubin in the blood and body tissues because bilirubin is one of the by-products of red blood cell destruction. However, the fact that there were relatively few children with jaundice implies that haemolytic process resulting in jaundice manifestation was relatively minimal. Some previous studies have demonstrated the significant role that malaria parasites have in red blood cell destruction thus causing anaemia, although the predominant pathogenetic mechanism is incompletely understood (Abdalla *et al*., 1980). Persistently low parasitaemia is a well recognized cause of chronic anaemia in African children, but the low parasitaemia responds rapidly to anti-malarial treatment (Kitua *et al*., 1997). In African children, the severity of malaria-associated anaemia is not often proportional to the degree of parasitaemia found at disease presentation but rather, the anaemia may result from the effect of cumulative parasite densities one to three months after infection with malaria parasites (McElroy *et al*., 2000; Ekvall *et al*., 2001).

Results of the current study showed no significant relationship ($p>0.05$) between malaria parasite density and hepatomegaly, splenomegaly and actual haemoglobin levels of affected children. However, splenomegaly was most frequently associated with moderate to severe anaemia among the children with malaria. Splenomegaly may be a common feature of malaria infection and is used as a crude indicator of malaria endemicity. A study done in the malaria-endemic lowlands of Western Kenya by Obonyo *et al*., (2006) found that splenomegaly was an independent risk factor for malaria-related anaemia. This

was also consistent with findings from Zaire and Thailand (Herdberg *et al*., 1993; Price *et al*., 2001). A study in Saudi Arabia by Annobil *et al*., (1994) reported that most patients with malaria fever also had enlarged liver and spleen. The spleen has an important pathophysiological role by removing parasitized and non-parasitized red cells from the individual infected with malaria (Chotivanich *et al*., 2000). However, there were relatively few cases of splenomegaly and hepatomegaly from the current study. The relatively few children with splenomegaly and hepatomegaly may relate to semi-immune response to malaria common in epidemic malaria-prone highland areas (Chotivanich *et al*., 2000). This therefore suggests that acute malaria, especially within the epidemic malaria-prone highland region of Western Kenya may not immediately trigger immune response to the degree of causing hepatomegaly and/or splenomegaly among children.

From the time of their introduction, malaria rapid diagnostic tests were not well received by the intended implementers. Consequently some MRDT formulations were withdrawn while others were modified by their manufacturers. Inconsistency in manufacturing standards, quality control problems and variable product stability also compounded the utilization of MRDT products (Mason *et al*., 2002; Shiff *et al*., 1993). Furthermore, studies assessing perception of patients or care-takers (Chandler *et al*., 2008; Tarimo *et al*., 2000) and clinicians (Polage *et al*., 2006; Ngasala *et al*., 2008) with respect to malaria diagnosis have elaborated among other things two important scenarios. One is that patients prefer laboratory testing before anti-malarial drug prescription and the other is that sometimes malaria laboratory results may have little influence in prescription practices by clinicians. The reasons behind underutilization of malaria laboratory results by clinicians needed to be investigated and addressed accordingly in order to build a

culture of rational management of malaria. Within the context of this knowledge gap, a questionnaire was designed for the current study to compare various responses from the health professionals working either in the epidemic malaria-prone highlands or malaria-endemic lowlands of Western Kenya.

From the responses to the questionnaires distributed as part of the current study, it is worrying to realize that a high proportion of professionally eligible health professionals from the epidemic malaria-prone highlands of western Kenya had not been trained to use malaria rapid diagnostic tests. Although the situation is better in the malaria-endemic lowlands of western Kenya because slightly less than a quarter of the eligible health professionals indicated they had never been trained, the overall highly significant discrepancy in the training proportions within the epidemic malaria-prone highlands and malaria-endemic lowlands is a major concern. The results from the current study also showed that according to informed individual opinions of health professionals from both the highlands and lowlands, a higher percentage of health professionals from both the epidemic malaria-prone highlands and malaria-endemic lowlands responded that those who had been trained to use rapid diagnostic tests were comparatively fewer than those who responded otherwise. The implication of these findings is that as far as malaria diagnosis using rapid diagnostic tests is concerned, there is a serious problem, especially in the epidemic malaria-prone highland regions of western Kenya. The health professionals from the latter (highlands) were less sensitized or trained on the rapid diagnostic tests than the former (lowlands). It is also of grave concern that the vast majority of the health professionals at the operational level who are ultimately the actual implementers of the whole rapid diagnostic technology were neither trained nor

sensitized about the importance of the diagnostic technology. The success or failure of such an important diagnostic technology largely depends on how it is handled at the operational level. In the Kenyan situation therefore, there is an urgent need to evaluate the entire rapid diagnostic technology, especially at the operational-level implementation stages; this is mandatory if the entire rapid diagnostic technology is to be embrased and is expected to succeed in future.

According to published studies, the implementation of malaria rapid diagnostic tests (MRDT) has so far not fully succeeded in some places (Hamer *et al*., 2007). Furthermore, the trust by health professionals on the introduction of rapid diagnostic tests (MRDT) has been disappointing in other regions (Reyburn *et al*., 2007). The real reasons for the failed implementation, no matter how insignificant, should be established. Our study found that negative attitude towards malaria rapid diagnostic tests was partly due to lack of appropriate training and sensitization about the importance of the relatively new malaria diagnosis technology. Whereas it has been argued elsewhere that the improvement in diagnostic sensitivity alone may not translate into improved patient care (Duffy and Fried, 2005), a study carried out in Tanzania proposed that training of clinicians and appropriate supportive supervision may possibly change diagnostic behaviour of the clinicians (Masika *et al*., 2006). In the Kenyan situation therefore, the glaring disproportion in the regional training of health professionals on rapid diagnostic tests and lack of the sensitization that often goes with such training may partly have contributed to the reluctance of health professionals to regularly use malaria rapid diagnostic tests in western Kenya.

The epidemic malaria-prone highland regions of western Kenya has for a long time been prone to outbreaks of malaria, a situation that has caused many deaths, especially among children (Some, 1994; Oloo *et al*., 1996; Malakooti *et al*., 1998; Snow *et al*., 1999; Shanks *et al*., 2000). This is mainly because unlike the population in the malaria-endemic lowland regions, the epidemic malaria-prone highland population is less immune to malaria attacks (English *et al*., 2004; English *et al*., 2004; Bhattacharya & Manesh, 1998). The widely held reason for this scenario is that the transmission of *P. falciparum* in communities living within the highlands is limited primarily by low ambient temperature and that small changes in temperature could therefore provide transiently suitable conditions for unstable transmission within populations that have acquired little functional immunity (Lindsay & Martens, 1998). Furthermore, the epidemic malaria-prone highland of Kenya is densely populated and therefore merits special attention for malaria surveillance and management preparedness (Ministry of Health guidelines for malaria epidemic preparedness, 1999; Ministry of Health National Malaria Strategy: 2001-2010). In immune individuals living in malaria endemic zones (such as the malaria-endemic lowlands of Western Kenya), malaria parasitaemia may not be accompanied by fever or other symptoms of malaria and that even mild malaria parasitaemia can be rapidly fatal in non-immune individuals. Semi-immune populations tend to get high malaria parasitaemia levels after only a short duration of malaria infection because of insufficiently low level of antibodies against malaria, unlike the more immune populations found in endemic areas, where malaria at one time killed up to 15-20% of children aged five and below (Macleod, 1987). Prompt and accurate diagnosis of malaria, especially in areas known to be endemic to malaria has increasingly been

emphasized over the years (McKenzie *et al*., 2003; Reyburn *et al*., 2007). The World Health Organization particularly has emphasized that laboratory diagnosis of malaria is mandatory if success has to be realized in malaria management and control (WHO, 2006). New malaria diagnostic methods, which include malaria rapid diagnostic tests among others, have therefore become increasingly important in malaria diagnosis. Another improvement on malaria diagnosis was made by the Centers for Disease Control (CDC) personnel who have developed a portable, battery-powered light source for field microscopy that has proven very useful in field trials (CDC, 2006). Polymerase Chain Reaction (PCR) has also been used but it is also subject to technical limitations because it is expensive, and requires standardized protocols and validation of the specific protocol used in a given trial. For these reasons, PCR is not generally accepted as the "gold standard" for malaria diagnosis (Murray *et al*., 2008). Microscopy method therefore remains the gold standard for malaria diagnosis. This fact was emphasized during the current study because most health professionals, especially among the senior cadres from both the epidemic malaria-prone highlands and malaria-endemic lowlands commented in their responses that microscopy should still be considered the most reliable method of malaria diagnosis. Although malaria diagnosis by use of microscopic examination of malaria parasites remains the golden standard of malaria diagnosis, the microscopy method of diagnosis continues to have challenges not only in Kenya but in many other parts of the developing world. Inaccuracy in reading malaria parasite slides occurs commonly; sometimes even when this is done by well-trained laboratory experts (Amexo *et al*., 2004; English *et al*., 2004; Reyburn *et al*., 2004). The alarming situation currently faced by health professionals working in areas prone to malaria outbreaks, such as the

epidemic malaria-prone highlands of western Kenya, is the difficulty of detecting malaria parasites during the microscopic examination of thick and thin blood smears to diagnose malaria. However, nearly all peripheral health facilities rely entirely on the method (CDC, 2002).

The results from the five randomly selected rural health centres in Western Kenya epidemic malaria-prone highland region demonstrated that there was a significant difference in the clinically diagnosed and the laboratory confirmed malaria. Consequently a much higher proportion of patients were presumptively treated for malaria compared to those who were treated after laboratory confirmation of malaria. The fact that about 60% of the patients were presumptively treated for malaria implies that most of this proportion was not evaluated in the laboratory for possible malaria; a scenario that is very dangerous for children, especially those with associated anaemia. Furthermore, the laboratory results from 132 children with malaria at Kapkangani health centre showed that 85.6% of the children also had malaria-associated anaemia. Presumptive malaria treatment therefore has the potential of aggravating anaemia burden, especially among children with malaria because presumptively (in other words clinically) treated children would most unlikely be sent by the attending clinician, for laboratory evaluation. The pattern of the results was similar in all the selected health centres and is probably a true picture of the real scenario in most, if not all rural health centres in Western Kenya epidemic malaria-prone highlands and elsewhere.

Besides increasing the risk of malaria-associated anaemia burden, presumptive treatment of malaria also subjects the affected patients to expensive drug treatment regimens, such as the artemisinin combination therapy. Inappropriate use of such drugs may also

increase chances of drug resistance by malaria parasites. Furthermore, a lot of drugs are most likely wasted on patients who are suspected but not confirmed to actually have malaria by laboratory diagnosis. Ultimately, the consequences of malaria management among children in the highland region will be adverse, unless deliberate efforts to reduce presumptive malaria treatment are made. In other words, one of the benefits of reliance on confirmed malaria prior to its treatment is reduced overall cost of the treatment, because drug wastage on unnecessary presumptive treatment will be curtailed. Although clinical practitioners in malaria-endemic areas may justify that they presumptively treat malaria owing to its risk of causing mortality among children as evidenced by past malaria epidemics in the highland region of western Kenya (Some, 1994; Chandy *et al*., 1997), deliberate efforts to ensure that only laboratory-confirmed malaria is treated with anti-malaria drugs will ultimately reverse the adverse effects of presumptive malaria treatment. In this regard, the introduction of additional cost-effective laboratory diagnostic methods, such as the introduction of well-standardized rapid diagnostic tests to be handled by competently trained laboratory personnel will be a step in the right direction. A mechanism to ensure that regular evaluation of newly introduced malaria diagnosis methods should then be put in place for the benefit of future services if problems cited in previous studies (Murray *et al*., 2008) are to be overcome.

Similarly, the burden of anaemia on children may increase due to increase in malaria-associated anaemia if presumptive malaria treatment by clinical practitioners is not curtailed. In this regard, the recommendations from our current study concur with recommendations from some of the previous studies, which emphasized that because there had been relatively fewer data on test performance of rapid diagnostic tests on

children as regards the study of febrile illnesses **(**Gasser *et al*., 2005)**,** there was therefore a need to resolve the issue that concerns the diagnostic testing of young children with clinical illnesses resembling malaria (Murray *et al*., 2008). The challenges faced by health professionals therefore have a bearing not only in in malaria diagnosis but in the overall management of malaria. Furthermore, previous research found that people living in areas where malaria transmission is low and unstable remain at risk of clinical attacks of malaria at older ages, and epidemics can result in increased morbidity among school children, more deaths, and considerable disruption of schoolwork (Some, 1994).

There is need therefore, to rely more on evidence-based management of malaria in order to save on the meager resources that Kenya is at pains to distribute to all sectors of her economy. The problem of malaria diagnosis is however not unique to the developing countries. In developed countries and in regions where malaria is not endemic these problems are exacerbated by the fact that the expertise and/or experience of laboratory personnel in such places is often lacking because malaria is rare in such places (Bell & Peeling, 2006; Reyburn *et al*., 2007). A study in Ghana showed that obstacles that affect laboratory malaria diagosis are common in areas where malaria is endemic (Polage *et al*., 2006) and similar constraints have also been reported elsewhere in sub-Saharan Africa (Bates *et al*., 2004; Petti *et al*., 2006). Various studies in Tanzania (Ishengoma *et al*., 2009; Ishengoma *et al*., 2010) and Uganda (Nankabirwa *et al*., 2009) also reported that lack of good laboratory facilities could adversely affect proper management of malaria. The observations from our study agree with those from other studies elsewhere (Barat *et al*., 1999; Chandler *et al*., 2008) that the clinicians' perceptions and practice as pertains the optimal laboratory testing remains one of the major barriers to effective laboratory

use. Among the conclusions from the studies were the need for future trainings on the use of various rapid diagnostic tests and use of better staining techniques, especially the use of Giemsa staining technique, besides the introduction of other non-malaria diagnostics (Duffy & Fried., 2005; Masika *et al*., 2006). Regualar evaluations of malaria diagnosis and management practices in Kenya are therefore essential for the sake of better disease diagnosis and subsequent management.

Results of the current study show that in both the epidemic malaria-prone highland and malaria-endemic lowland regions, majority of the health professionals who had been trained to use malaria rapid diagnostic tests had been trained within five years from the time our study was carried out (from 2005 onwards), compared to the period before 2005. The fact that malaria rapid diagnostic use is a relatively new technology in western Kenya should be an encouragement in that many avenues to improve its use in the region are yet to be explored and evaluated. It is clear from the data of the current study that the training on the use of rapid diagnostic tests has so far not been uniform across the epidemic malaria-prone highlands and malaria-endemic lowlands. The health professionals from the epidemic malaria-prone highlands of western Kenya particularly require more training opportunities if the impact of using malaria rapid diagnostic tests in the region is to be realized. However, the Kenyan health professionals in the epidemic malaria-prone highlands of western Kenya should also be encouraged by a survey conducted earlier to explore the use of MRDT in United Kingdom laboratories. The UK survey found an overall survey response rate of 60.3%, whereby MRDT were the preferred choice, either alone or in conjunction with microscopy (Chilton *et al*., 2006).

Therefore, not all health professionals, including those in developed countries like the United Kingdom accepted the rapid diagnosis technology from its inception.

According to previous studies (Bell & Peeling, 2006; WHO, 2000) there are specific factors that need to be considered in introducing a rapid diagnostic test; among the important ones are performance characteristics, operational characteristics, and cost. Frequent evaluation on the use of rapid diagnostic tests should also have been a priority during the early stages of its implementation, whereby the apparent disproportion in training in western Kenya on the use of rapid diagnostic tests should have been detected much earlier. Such evaluation is important, considering that the attitude of health professionals towards the use is bound to definitely influence the success or failure of such a new technology. Although the health professionals in the current study who had been trained to use rapid diagnostic tests had been exposed to trainings on various types of the malaria diagnostic tests during their initial training, majority of them had been trained using ParaSight F and Immunochromatographic tests. The results of the current study also showed that there were varied responses from health professionals whether or not to recommend on the use of rapid diagnostic tests for malaria diagnosis. Whereas just over half of the health professionals from both the epidemic malaria-prone highlands and malaria-endemic lowlands recommended rapid diagnostic tests in addition to microscopic examination, majority of the remaining proportion were not sure whether or not to recommend their use. However, different sets of data from the current study confirmed that anywhere between sixty and eighty five percent of the health professionals from the epidemic malaria-prone highlands have so far not been trained to use malaria rapid diagnostic tests. Such a high proportion of eligible but untrained health professionals

cannot currently be relied upon to recommend or not to recommend the use of rapid diagnostic tests. Even if the user instructions of the rapid diagnostic tests are easy to understand and apply, sensitization on their importance is still needed and such sensitization is more often than not given by the experts during the initial trainings. Emphasis therefore should be to train a higher proportion of the health professionals and the training should be followed by regular evaluations on the impact of the training on the outcome of their use by various health professionals.

Malaria rapid diagnostic tests have been used experimentally for many years, initially in Thailand (Banchongaksorn *et al*., 1996; Stephens *et al*., 1999), the United Republic of Tanzania (Shiff *et al*., 1994); hence they are increasingly being considered for routine use in many African countries (Lubell *et al*., 2008), such as Botswana, Mozambique, South Africa, Swaziland and Cambodia . Kenya therefore has every reason to also try and embrace the malaria rapid diagnostic technology and to eventually roll it out on a wide scale countrywide. Evaluations of various rapid diagnostic tests have also been done in Médecins Sans Frontières (MSF) and Cambodia; both MSF experience and that from Cambodia indicate that large-scale confirmation of clinical malaria by MRDT is feasible, although difficulties of procurement, distribution, transport and storage need to be addressed (Guthmann *et al*., 2002; WHO, 2006). The use of rapid diagnostic tests has also been recommended for many other parts of Sub-Saharan Africa (Snounou & White, 2004). Although most MRDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria (Lee *et al*., 2008; Park *et al*., 2006; Kim *et al*., 2008). This is bound to be useful information to health professionals during the intial trainings on the use of malaria rapid diagnostic tests.

Results from our study showed that about sixty percent of the questionnaire respondents from the epidemic malaria-prone highland region declined to recommend any preferred rapid diagnostic test for malaria diagnosis. Such a high proportion of health professionals who declined from recommending any of the rapid diagnostic tests reaffirmed the overall lack of essential information necessary for successful and reliable evaluation of the relatively new malaria diagnostic technique. It also emphasizes why the health policy makers in Kenya (particularly in health institutions in Western Kenya) and elsewhere, should re-double their efforts to train more health professionals, if successful implementation of the relatively new technology is to be realized.

Unlike in the epidemic malaria-prone highlands, the highest proportion of the health professionals who positively responded from the malaria-endemic lowlands belonged to the middle-level laboratory cadre (laboratory technologists and technicians) followed closely by the middle-level clinical cadres (mainly nurses, clinical and public health officers). A higher proportion of health professionals from the senior cadre participated in the malaria-endemic lowlands than in the epidemic malaria-prone highlands. It was also noted that most of the senior cadre personnel (mainly doctors and research scientists) who participated recommended that microscopy remains the gold standard technique for malaria diagnosis. It is important to note that the additional comments about microscopy as the gold standard during the current study came mainly from the senior health personnel. The training background of the health professionals is another challenge to be faced in the implementation of the relatively new rapid diagnostic technique of malaria. The senior cadre of health professionals in the current study were those with University-level training in health-related courses, while the middle cadre were those with diploma

or certificates in various health-related courses. The scope of training of health professionals may therefore influence their responses in such a study and perhaps, their overall attitude to such new diagnostic techniques. These findings are consistent with those in a recent study conducted in Tanzania by Yahya *et al*., (2011), which found that the quality of malaria diagnosis and management was adversely affected by inadequately trained personnel, some of whom lacked professional qualifications.

The overall percentage gender distribution of health professionals who responded to the questionnaire from both the epidemic malaria-prone highland (46.7%) and malaria-endemic lowland (53.3%) regions was approximately equal. This implies that there was approximately the same percentage of men and women who were on duty at various healthcare institutions when the study was carried out and who willingly participated in the study. However, unlike in the malaria-endemic lowland region, there were relatively a higher percentage of female health professionals in the epidemic malaria-prone highlands (72.1%) than the males (60.6%) who responded positively to the study. The statistically significant difference in gender distribution was probably due to the much higher population of respondents from the epidemic malaria-prone highlands than the malaria-endemic lowlands.

Results of the current study revealed anaemia among 85.6 % of the children with malaria whereby 54.6% and 23.5% had moderate and severe anaemia, respectively. It was established from the results that over 96% of the anaemia was due to *Plasmodium falciparum*. This was consistent with the previous research findings, which implicated *Plasmodium falciparum* to account for over 90% of malarial infections (Bloland *et al*., 1999). Majority of the children considered for the current study therefore had malaria-

associated anaemia. The finding from the study supports results from a previous research whereby it was reported that up to three quarters of African children are estimated to have anaemia (haemoglobin caused mainly by malaria or iron deficiency (DeMaeyer & Adels-Tegman, 1985). Although the aetiology of anaemia in Africa is multifactorial, infection with *Plasmodium falciparum* (Brooker *et al*., 1999; Geissler *et al*., 1998) and anti-malarial resistance (Bloland *et al*., 1993; Ekvall *et al*., 1998) are considered among the commonest causes, especially among children. Other previous studies elsewhere have shown that malaria-associated anaemia is a major cause of morbidity, admission and mortality among children, especially in Sub-Saharan Africa. However, this scenario is worrying because other previous studies elsewhere have shown that malaria-associated anaemia is a major cause of morbidity, admission and mortality among children, especially in Sub-Saharan Africa. Anaemia is often unrecognized and undertreated since it often presents with non-specific signs and symptoms (Schellenberg *et al*., 2003). If left untreated, anaemia is a major risk factor that might cause mortality (Mabeza *et al.*, 1998). Although severe anaemia due to malaria more often occurs in areas with high malaria transmission, such as the malaria-endemic lowlands of Western Kenya (Obonyo *et al*., 2003), the results from the current study suggest that severe anaemia should also be expected in the epidemic malaria-prone highland areas where malaria transmission has been known to occur mainly during the rainy seasons. Thus, to be on the safe side, healthcare professionals should consider anaemia management along with malaria management among children. Other causes of anaemia established from previous researches include human immunodeficiency (Van Eijk *et al*., 2002; Otieno *et al*., 2006), intestinal helminthes (Brooker *et al*., 1999; Geissler *et al*., 1998), haemoglobinopathies

(Fleming & Werblinska, 1982), bacteraemia (Graham *et al*., 2000), poor nutritional status and micronutrient deficiencies (Verhoeff *et al*., 2002; Nussenblatt & Semba, 2002) among others. Malaria-related anaemia affects an estimated 1.5 to 6 million African children and is responsible for a case fatality rate of 15% (Murphy & Breman, 2001).

Analysis of the data for haemoglobin and malaria parasite density from the current study showed a negative Pearson's correlation between the actual haemoglobin levels and malaria parasite density, although the correlation was statistically insignificant (Pearson's correlation coefficient 'R'= -0.1171; p = 0.181). The explanation for the negative correlation between haemoglobin levels and malaria parasite density among the children with malaria is that as malaria infection becomes more intense or severe; more red blood cells are broken down (haemolysed). The implication of this finding is that the higher the density of malaria parasites, the more the red blood cell destruction by the malaria parasites. Anaemia due to malaria is thought to develop through increased destruction or reduced production of red blood cells or a combination of both processes. However, the predominant pathogenetic mechanism is incompletely understood (Abdalla *et al*., 1980). Persistently low parasitaemia is a well recognized cause of chronic anaemia in African children, but the low parasitaemia responds rapidly to anti-malarial treatment (Kitua *et al.*, 1997). In African children, the severity of anaemial anaemia is not often proportional to the degree of parasitaemia found at disease presentation but rather, the anaemia may result from the effect of cumulative parasite densities over the past one to three months (McElroy *et al*., 2000; Ekvall *et al*., 2001). Failed malaria treatment contributes to malaria-associated anaemia resulting from persistence of malaria parasitaemia that consequently has been responsible for increased hospitalization and blood transfusion

(Greenberg *et al*., 1988; Zucker *et al*., 1996). Hence the noted correlation of malaria parasitaemia with malaria parasite density in the current study should be considered to be of paramount importance because if malaria parasitaemia is not controlled in time, it may contribute to the need for blood transfusion. In some places, 20-50% of children admitted for malaria were transfused (Greenberg *et al*., 1988; Lackritz *et al*., 1992). Blood transfusion however, has been associated with increased risk of transmitting immunodeficiency virus (HIV) among other related risk factors. In addition to the many transfusion risks, a high proportion of paediatric blood transfusions are inappropriately prescribed (McFarland *et al*., 1997: Moore *et al*., 2002). A number of studies have described the risk factors for malaria induced anaemia in Africa and Asia (Hedberg *et al*., 1993; Redd *et al*., 1994; Premji *et al*., 1995; Achidi *et al*., 1996; Luckner *et al*., 1998; Cornet *et al*., 1998; Price *et al*., 2001; Van Eijk *et al*., 2002; Kahigwa *et al*., 2002; Owusu-Agyei *et al*., 2002; Verhoeff *et al*., 2002: Schellenberg *et al*., 2003: Akhwale *et al*., 2004: Desai *et al*., 2005; Mulenga *et al*., 2005; Otieno *et al*., 2006; Ong'echa *et al*., 2006; WHO, 1968). The principal factors included the infecting malaria parasite species, intensity of malaria parasite transmission, patient's age, host-genetic factors and presence of other concomitant, non-malarial causes of anaemia. The pathogenesis of malarial anaemia is often multifactorial, complex and incompletely understood. Postulated mechanisms fall broadly under heamolysis and dyserthropoiesis.

Malaria-associated anaemia may present either as an acute episode or as a chronic process following repeated, often asymptomatic infection (McGregor *et al*., 1966; Phillips & Pasvol, 1992). The latter seems to apply to the epidemic malaria-prone highland study area where the current research was conducted because most of the

registered complaints affecting the children were fever associated with sweating and chills, vomiting and headache. A previous study found that sweating was among the clinical features commonly associated with malaria (Menendez *et al*., 2000). Although in the current study, anaemia turned out to have been the second commonest clinical feature, it was mostly asymptomatic and its discovery was more of an incidental finding than otherwise.

The results from our study partly demonstrated that malaria prevalence has reduced over the years, as seen from the data and illustrations on malaria diagnosis from some of the rural health centres selected for the study. This confirms what was earlier reported from a study, which showed that epidemiology and risk of malaria in Kenya are declining (Division of Malaria Control, Kenya, 2011). A study in one epidemic-prone zone of western Kenya attributed the dramatic reduction of malaria parasite transmission to widespread implementation of malaria control interventions such as insecticide-treated bed nets (ITNs) and artemisinin combination therapy (ACT) that (Lindblade *et al*., 2004; Bhattarai *et al*., 2007; Okiro *et al*., 2007) have been introduced in western Kenya in the recent past. For the first time since the 1950s, the World Health Organization and other organizations are promoting malaria eradication (Greenwood *et al*., 2008). In epidemic malaria-prone highland areas (>1,500 m above sea level) in Africa, malaria transmission is unstable, with a low incidence of malaria during dry seasons (Ernst *et al.*, 2006). These areas may be ideal initial targets for attempting the interruption of malaria transmission. Also in these areas, the combination of ACT, which may decrease transmission by reducing gametocyte load in infected persons, and annual indoor residual spraying (IRS)

with long-lasting insecticides, which can reduce indoor vector density for a prolonged period, could act synergistically to interrupt malaria transmission.

The epidemiology of malaria over small areas remains poorly understood, and this is particularly true for malaria during epidemics in epidemic malaria-prone highland areas of Africa, where transmission intensity is low and characterized by acute within and between year variations (Brooker *et al*., 2004). In Kenya, the health centres and dispensaries handle most of the country's population, despite such facilities being less equipped in terms of material, manpower resource and expertise. It is in such humble healthcare setups that malaria diagnosis is bound to be more challenging, given the scarce resources that the health professionals deployed to work in such health institutions have at their disposal. In addition to the often high population of patients that seek medical attention in such peripheral health facilities, the ambiguous clinical picture of malaria, in relation to other common diseases that may simulate malaria (Makani *et al*., 2003; Molyneux & Koram, 2007) pause extra diagnosis challenges. From this perspective therefore, the current study sought to evaluate the diagnostic and treatment practices in peripheral healthcare institutions. Five health centres situated in the epidemic malaria-prone highlands of western Kenya were therefore targeted for the study.

The period prevalence of malaria in two health centres (Turbo and Kapkangani Health Centres) situated within the epidemic malaria-prone highlands of western Kenya where past published researches reported malaria epidemics, has consistently remained higher than all other common diseases in that region, within the period between 2005 and 2010. Turbo Rural Health Centre, which is situated within the epidemic malaria-prone highlands of western Kenya, has had many seasonal outbreaks of malaria (Some, 1994;

Akhwale *et al*., 2004; Shanks *et al*., 2004). It is important to note that malaria remains the most prevalent disease in Turbo to date. Malaria also remains the most prevalent disease in other regions immediately adjacent to Turbo and Kapkangani locations, both of which are situated within the highlands of western Kenya, whereby in the past, notable malaria outbreaks have occurred (Arness *et al*., 2003; Brooker *et al*., 2004; Shanks *et al*., 2004; Kacey *et al*., 2006; Muller *et al*., 2009; Chandy *et al*., 2009).

. However, the prevalence of malaria reduces in areas placed at a higher altitude, such as the areas closer to Eldoret town (2100metres above sea level), as shown by the data from three health centres situated in separate campuses of Moi University. Results from each of the three other health centres situated within Moi University Campuses around Eldoret (Chepkoilel, Town campus and Main Kesses campus health centres respectively) also showed that the period prevalence of malaria was second, after upper respiratory infections. However, upper respiratory infections are more common than malaria in the epidemic malaria-prone highland region where Moi University campuses near Eldoret town are situated most likely because of climatic changes influenced by the nearby Mau Range of the Great Rift Valley Escarpment. Furthermore, the term 'upper respiratory tract infections' signifies a wide range of diseases, most of which are associated with seasonal environmental changes occasioned by the geographical and climatic conditions already mentioned above, given that most of the affected people reside close to the nearby Mau Range of the Great Rift Valley escarpment. The collective prevalence of all of them is therefore bound to be higher compared to a single disease: malaria. Hence malaria may still be the most prevalent disease compared to each of the separate respiratory diseases collectively classified as 'upper respiratory tract infections'.

In all the three health centres, malaria was the second most prevalent disease across several years. The fact that malaria prevalence occupies first or second place within the epidemic malaria-prone highlands of western Kenya implies that despite the reported drastic reduction in malaria as a result of the implementation of the indoor residual spray (IRS) intervention measures (Chandy *et al*., 2009), new approaches to control or eradicate malaria should be sought and implemented; hence it may be too early to become complacent or celebrate the successful war against malaria.

From the results of the current study, a notable general observation in the diagnosis of malaria across the years as far as the selected rural health centres were concerned was the general reduction in the total number of patients evaluated for possible malaria. This was observed across the selected health centres and within the various health centres in the epidemic malaria-prone highland region. The major reason that has caused drastic reduction in malaria burden in many parts of the epidemic malaria-prone highland region of western Kenya is the successful intervention measures occasioned by the the annual indoor residual spraying (IRS) with long-lasting insecticides, which can reduce indoor vector density for a prolonged period, in addition to the artemisinin combination therapy that act synergistically to interrupt malaria transmission (Lindblade *et al*., 2004; Barnes *et al*., 2005; Bhattarai *et al*., 2007; Okiro *et al*., 2007; Chandy *et al*., 2009). It is notable and appreciable that for the first time since 1950s the World Health Organization and other organizations have began promoting eradication of malaria (Greenwood *et al*., 2008). A much larger study in South Africa in which IRS treatment and ACT treatment of persons with clinical malaria were introduced sequentially demonstrated an additional reduction of malaria incidence after introduction of ACT (Barnes *et al*., 2005) and this

supports the idea of synergy between the two intervention methods against malaria. The Roll Back Malaria program (WHO, 2008) and the study by Chandy *et al*., (2009) currently recommend IRS as the preferred method of reducing malaria in areas of low transmission. Since our study area (Nandi County) is one of the current beneficiaries of the IRS programme the initial results of the IRS programme in this area are encouraging.

It is also possible that unpredictable weather changes occasioned by global warming may have caused drastic global weather changes over the years (Loevinsohn, 1994; McMichael, 1997; Snow *et al*., 1998; Githeko & Ndegwa, 2001; Zell, 2004) culminating in prolonged drought and thus interrupting malaria transmission in some areas. However, global warming and other adverse effects on the environment, such as deforestation of the Mau range and other forests may also have increased the incidence of malaria in some parts of Kenya, such as the Central Kenya Epidemic malaria-prone highlands near Mt. Kenya (Githeko *et al*., 2000; Nabil & Qader, 2009). In Kenya the weather changes have at times caused unusual floods that occurred in many parts of the country that typically experienced long dry climatic spells.

Another general finding from our study, concerning malaria diagnosis within and across various selected rural health centres was that the statistically significant proportion of presumptively treated patients was much higher compared to those microscopically confirmed to have malaria parasitaemia. The implication of this finding is that more patients were treated on basis of clinical malaria features than those treated on basis of laboratory confirmation of malaria parasitaemia, especially in rural health centres within the epidemic malaria-prone highland region of Western Kenya. This may translate to

enormous amounts of financial and other resources spent on the management of malaria diagnosed and subsequently treated on the basis of clinical rather than laboratory diagnosis; a challenge that is experienced in nearly all peripheral health institutions because they rely on microscopic examination of thick and thin blood smears for malaria diagnosis (CDC, 2002). Since this problem is not unique to Kenya and that it affects many countries in Sub-Saharan Africa, it was recommended that such countries should reassess their malaria diagnosis practices (Makani *et al*., 2003).

The economic implication of malaria diagnosis is especially important when it is considered that the current recommended artemisinin-based combination therapy (ACT) regimens of malaria drugs are very expensive. Self-prescribed anti-malarial and anti-pyretic drugs also have the potential of masking or delaying the diagnosis of diseases that may present with fever. Furthermore, overdiagnosis of malaria implies underdiagnosis and inappropriate treatment of non-malarial febrile illness, such as the potentially fata bacterial meningitis, some viral diseases, respiratory infections (Brent et al., 2006) and typhoid (Reinherdt et al., 1978) among others.

A survey done in Tanzania to establish the accuracy of malaria microscopy in some health facilities (Ishengoma *et al*., 2010) revealed that the ability of the laboratory personnel in detecting malaria infection by microscopy was approximately fifty percent (Ngasala *et al*., 2008). This implies that most of the patients reported to have had malaria parasites either did not actually have the parasites or the micropists missed to see the parasites that actually existed in the blood sample. The reported relatively high malaria slide positivity rate coupled with the observed presumptive diagnosis practices indicates

high level of malaria misdiagnosis in the study health facilities. Apart from the significantly low sensitivity of microscopy method of malaria diagnosis, there are other challenging problems associated with it. The staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained health professionals, particularly for identifying species accurately at low parasitemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasitaemia levels, when parasites are sequestrated or when malaria parasite viability is impaired by chemotherapy. As a result of this shortcoming, highly sensitive detection of malaria cannot be achieved with Giemsa-stained thick and thin smears, for purposes of screening malaria from blood transfusion donors (Kitchen & Chiodini, 2006). Examination of serially obtained smears helps to overcome the challenges posed by parasite sequestration and initially low parasite densities (Hanscheid, 1999). Microscopy diagnostic errors are noted more commonly for low-density parasitemias. In addition, mixed infections are often missed, especially when *P. malariae* or *P. ovale* parasites are present, as their densities are often low in comparison to that of *P. falciparum*. Finally, even when the test is ordered, clinicians may choose not to wait for the test results or may lack confidence in the test result and treat the patient despite negative microscopy (Hamer *et al*., 2007; Reyburn *et al*., 2007). These problems are exacerbated in regions where malaria is not endemic, as malaria microscopy is performed infrequently. Illustrating this problem, a study of 100 patients in

Although the expert microscopist can detect between 5-10 parasites per microlitre of blood, the average microscopist detects only 50-100 parasites per microlitre of blood

(Murray *et al*, 2008; Payne, 1988). This has probably resulted in under-estimating malaria infection rates, especially cases with low parasitemia and asymptomatic malaria. Furthermore, an individual microscopist's expertise in interpreting blood slides can diminish over time (Murray *et al*., 2008). The ability to maintain required levels of accuracy in malaria diagnostics expertise is problematic, especially in remote medical centers in countries where the disease is rarely seen (Ohrt *et al*., 2002). Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density is still challenging. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable (Erdman & Kain, 2008).

Whereas it is appreciable that health professionals in malaria-prone areas encounter numerous challenges in the diagnosis process of diseases that present with fever or other complex of diseases that may coexist with malaria (Sowunmi & Akindele, 1993; Schellenberg *et al*., 1994; Luxemburger *et al*., 1998; Chandramohan *et al*., 2002; Arness *et al*., 2003; Peters *et al*., 2004; Reyburn *et al*., 2004; Taylor *et al*., 2004; Duffy & Fried, 2005; Berkley *et al*., 2005; Brent *et al*., 2006; Hamer *et al*., 2007; Ndyomugyenyi *et al*., 2007; Bhandari *et al*., 2008), it should be noted that indiscriminate use of anti-malarials can seriously compromise the quality of care for patients with non-malarial fevers in malaria-endemic areas (Berkley *et al*., 2005; Mwangi *et al*., 2005; Reyburn *et al*., 2004; McMorrow *et al*., 2008). Unfortunately and regrettably so, malaria control in tropical Africa has principally been based on the presumptive treatment of fever using anti-malarial drugs (Chandramohan *et al*., 2002; Trape *et al*, 2002). Hence, the recommendation by WHO is that the parasite-based diagnosis should be used in all cases

of suspected malaria with the exception of among other situations, children in high-malaria prevalence areas (WHO, 2008).

The proportion of children from the current study who had already been given anti-malarial drugs at home prior to hospital treatment was 8.3%. The results from the current study therefore demonstrated that some children were subjected to self-prescribed anti-malarial drugs; a practice that should be discouraged if drug resistance in malaria management is to be contained. Another known major risk of self-prescribed anti-malarial drugs is the development of drug resistance, which has been found to contribute to the increasing prevalence of malaria-associated anaemia that is also partly attributable to malaria-related mortality in African children (Hedberg *et al*., 1993; Slutsker *et al*., 1994; Trape *et al*., 1998; Bjorkman, 2002). One of the consequences of drug resistance is poor haematological recovery from malaria (Bloland *et al*., 1993; Verhoeff *et al*., 1997; Ekvall *et al*., 1998). The complex problem of drug resistance in malaria treatment has compelled the World Health Organization to recommend the use of artemisinin-based combination therapy (ACT) as the standard care in the treatment of uncomplicated *Plasmodium falciparum* malaria (WHO, 1998). However, the introduction of ACTs has brought about new challenges of serious limitations of ACT drug availability, familiarity and affordability by users (Bloland, 2003). It is appreciable that in Kenya, the efficacy of anti-malarials continues to be monitored every two years, since Kenya adopted the artemisinin based combination treatment (ACT) artemether-lumefantrine (AL) as first line treatment for uncomplicated malaria in 2006. The second line treatment is dihydroartemisinin-piperaquine (DHA-PPQ). The recommended treatment for severe malaria is parenteral quinine or artemisinins where available. The corrected day 28

efficacy of AL at baseline in 2005 was 97.3 per cent and in 2007 the efficacy was 96.4 per cent (Obonyo *et al*., 2003; Ogutu *et al*., 2000; Adjuik, 2002; Elizabeth *et al*., 2008).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Immunochromatographic rapid diagnostic test is significantly sensitive and specific to *Plasmodium falciparum*, among children aged twelve and below.

There were no significant differences among the sensitivities of immunochromatographic malaria rapid diagnostic test using whole blood, plasma and serum specimens, respectively, for the diagnosis of malaria in children aged twelve and below. However, whole blood or plasma is more reliable than serum for malaria diagnosis.

There were significant differences in the training and user-attitudes of eligible health professionals in the highlands and lowlands of western Kenya towards malaria rapid diagnostic tests.

Anaemia is significantly prevalent among children with malaria in the highlands of western Kenya, where our study was carried out. However, there was a negative correlation between haemoglobin levels and malaria parasite density among the children with malaria, but the negative correlation was statistically insignificant.

Presumptive malaria treatment is significantly more practiced than laboratory diagnosis of malaria, especially in rural health centres considered for our study in western Kenya.

Incosistency in keeping up-to-date data about malaria morbidity was encountered in the five rural health centres considered for our study.

6.2 RECOMMENDATIONS

Immunochromatographic rapid diagnostic test should be used to support microscopy method of malaria diagnosis.

Although whole blood, plasma or serum is recommended for malaria diagnosis using immunochromatographic test, serum is less reliable than plasma or whole blood. Urgent training to sensitize qualified health professionals, especially in the highlands of western Kenya, to effectively use malaria rapid diagnostic tests, is vital.

Investigations to establish and manage the causes of the high anaemia prevalence among children (aged twelve and below) with malaria, are urgently required. Measures to reduce presumptive malaria treatment and increase reliance on laboratory diagnosis of malaria should be identified and implemented.

Measures to improve and update malaria morbidity statistics of children, to ensure consistent availability of malaria data in health facilities, are urgently required.

REFERENCES

Abdalla, S., Weatherall, D.J., Wickramasinghe, S.N., Hughes, M. (1980). The anaemia of *P. falciparum* malaria. British Journal of Haematology, 46**,** pp.171-83.

Abeku, T.A., Kristan, M., Jones, C., Beard, J., Mueller, D.H. (2008). Determinants of the accuracy of rapid diagnostic tests in malaria case management: Evidence from low and moderate transmission settings in the East African epidemic malaria-prone highlands. Malaria Journal, 7, P. 202.

Achidi, E.A., Salimonu, L.S., Asuzu, M.C., Berzins, K., Walker, O. (1996). Studies on *Plasmodium falciparum* parasitaemia and development of anaemia in Nigerian infants during their first year of life. American Journal of Tropical Medicine and Hygiene, 55, pp. 138-143.

Adjuik, M. (2002). Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial. *Lancet*, 359 (9315), pp. 1365-72.

Akhwale, W.S., Lurn, J.K., Kaneko, A., Eto, H., Obonyo, C., Bjorkman, A., Kobayakawa, T. (2004). Anaemia and malaria at different altitudes in the western epidemic malaria-prone highlands of Kenya. *Acta Tropica*, 91, pp.167-175.

Alserouri, A.W., S. M. Grantham-Mcgregor., B. Greenwood and A. Costello. (2000). Impact of asymptomatic malaria parasitaemia on cognitive function and school achievement of schoolchildren in the Yemen Republic.Parasitology, 121, pp 337-345.

Alves, F.P., Gil, L.H., Marrelli, M.T., Ribolla, P.E., Camargo, E.P., Da Silva, L.H. (2005). Asymptomatic carriers of *Plasmodium* spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. *Journal of Medical Entomology*, 42, pp.777–779.

Amexo, M., Tolhurst, R., Barnish, G., Bates, I. (2004). Malaria misdiagnosis: effects on the poor and vulnerable. Lancet, 238**,** pp.1896–1898.

Amstrong, S, J.R.M., Smith, T., Alonso,P. L., Hayes, R.J. (1994). What is malaria? Finding case definitions for field research in highly endemic areas. *Parasitology Today,* 10**,** pp. 439-442.

Annobil, H., Theodore, D., Ghazi, J., Wagih, A.(1994). Malaria in children-Experience from Asir region, Saudi Arabia. *In*: *Annals of Saudi Medical Journal*, 14 (6), pp.467-470.

Arness, M.K., Bradshaw, K.B., Shanks, G.D. (2003). Epidemiology of epidemic malaria-prone highland malaria in western Kenya. *East African medical journal*, Volume: 80, Issue: 5, pp: 253-259.

Babiker, H.A., Schneider, P. (2008). Application of molecular methods for monitoring transmission stages of malaria parasites. *Biomedical Mater*, 3**,** 034007.

Baker, J., J., McCarthy, M., Gatton, D. E.. Kyle, V., Belizario, J., Luchavez, D., *et al.* (2005). Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. *Journal of Infectious Diseases,* 192**,** pp**.** 870–877.

Banchongaksorn, T. (1996). A field trial of the ParaSight™–F test for the diagnosis of *Plasmodium falciparum* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 90**,** pp. 244–245.

Banoo, S., D. Bell, P., Bossuyt, A., Herring, D., Mabey, F., Poole, P. G., *et al.* (2006). Evaluation of diagnostic tests for infectious diseases: General Principles. *National Rev. Microbiology,* 4, S20–S32.

Barat, L., Chipipa, J., Kolczak, M., Sukwa, T. (1999). Does availability of blood slide microscopy for malaria at health centres improve the management of persons with fever in Zambia. *American Journal of Tropical Medicine& Hygiene*, 60, pp.1024-1030.

Barman, D., Mirdha, B.R., Samantray. J.C., Kironde. F., Kabra. S.K., Guleria, R. (2003). Evaluation of quantitative buffy coat (QBC) assay and polymerase chain reaction (PCR) for diagnosis of malaria. *Journal of Communicable Diseases,* 35, pp.170–181.

Barnes, K.I., Durrheim, D.N., Little, F., Jackson, A., Mehta, U., Allen, E., *et al.*, (2005). Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. PLoS Med. 2005, 2, e330.

Barnish, G., Bates, I and Iboro, J. (2004). 'Newer drug combinations for malaria may be impractical unless diagnostic accuracy can be improved' *British Medical Journal*, Vol 328, pp.1511-1512.

Bartoloni, A. (1998). False positive ParaSight®–F test for malaria in patients with rheumatoid factors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 92, pp. 33–34.

Bates I, Bekoe V, Asamoa-Adu A. (2004). Improving the accuracy of malaria-related laboratory tests in Ghana. *Malaria Journal*, **3**: P. 38.

Bates, I and Maitland, K.(2006). Are laboratory services coming of age in sub-Saharan Africa? *Clinical Infectious Diseases*, 42, pp. 383-384.

Beadle, C. (1994). Diagnosis of malaria by detection of *Plasmodium falciparum* HRP2 antigen with a rapid dipstick antigen-capture assay. *Lancet*, 343, pp. 564–568.

Bell, D.R., Wilson, D.W., Martin, L.B. (2005). False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. *American Journal of Tropical Medicine & Hygene*; 73, pp. 199 –203.

Bell, D., and **Peeling, R. W. (2006).** Evaluation of rapid diagnostic tests: malaria. *National Rev. Microbiology Journal*, 4 (Suppl. 9), S34–S38.

Bell, D., Wongsrichanalai, C., Barnwell, J.W. (2008). Ensuring quality and access for malaria diagnosis: how can it be achieved? *National Rev Microbiology.Journal*, 4, S7–S20, 682–95.

Behrens, R. H., and **C. J. Whitty. (2000).** Self-use of rapid tests for malaria diagnosis. Lancet, 355, P. 237.

Behrman, R.E., Kliegman, R.M., Jenson, B.H. (2004). *Nelson's Textbook of Pediatrics.*(17th ed). Saunders. P.1309.

Berkley, J.A., Maitland, K., Mwangi, I., Mwarumba, J. A. Scott, and M. English. *et al.* (2005). Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. *British Medical Journal*, pp. 330:995.

Berkley, J.A., Lowe, B.S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S. *et al.* (2005). Bacteremia among children admitted to a rural hospital in Kenya. *New English Journal of Medicine*, 352, pp. 39-47.

Bernard, P and Morrison. (eds.) (1991). Nursing Research in Action: Developing Basic Skill. Cardiff: MacMillan Publishers.

Bhandari, P.L., Raghuveer, C.V., Rajeev, A., Bhandari, P.D. (2008). Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. *Indian Journal of Pathology& Microbiology,* 51, pp.108–112.

Bharti, A.R., Patra, K.P., Chuquiyauri, R., Kosek, M., Gilman, R.H., Vinetz, J.M., *et al.* (2007). Polymerase chain reaction detection of *Plasmodium vivax* and *Plasmodium falciparum* DNA from stored serum samples: implications for retrospective diagnosis of malaria. *American Journal of Tropical Medicine & Hygiene*, 77, pp. 444–446.

Bhattacharya, P.C and Manesh, P.B. (1998). Unusual manifestations of pernicious malaria and newer diagnostic avenue. *In*: *Medicine Update.* APICON, vol.8, pp.156-63.

Bjorkman, A. (2002). Malaria-associated anaemia, drug resistance and anti-malarial combination therapy. *International Journal for Parasitology*, 32, pp.1637-43.

Bloland, P.B., Lackritz, E.M., Kazembe, P.N., Were, J.B.O., Stekettee, R and Campell, C.C. (1993). Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infections Diseases,* 167, pp. 932-7.

Bloland , P.B., Boriga, D.A., Ruebush, T.K., McCormick, J.B., Roberts, J.M., Oloo, A.J., *et al.* (1999). Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. American *Journal of Tropical Medicine and Hygiene*, 60, pp. 641-8.

Bloland, P.B., Kachur, S.P., Williams, H.A. (2003). Trends in antimalarial drug deployment in Sub-Saharan Africa. *Journal of Exp Biology*, 206, pp.3761–9.

Brent, A.J., Ahmed, I., Ndiritu, M., Lewa, P., Ngetsa, C., Lowe, B., *et al*., (2006). Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. *Lancet*; 367, pp. 482-8.

Brinkmann, U. and **Brinkmann, A. (1991).** Malaria and health in Africa: the present situation and epidemiological trends. *Tropical Medicine & Parasitology*, 42, pp. 204-13.

Brooker, S., Clarke, S., Njagi, J.K., Polack, S., Mugo, B. (2004). Spatial clustering of malaria and associated risk factors during an epidemic in a epidemic malaria-prone highland area of western Kenya. *Tropical Medicine International Health.* Volume: 9, Issue: 7, pp. 757-766.

Brooker, S., Peshu, N., Warn, P.A., Mosobo, M., Guyatt, H.I., Marsh, K., Snow, R.W. (1999). The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 93, pp. 240-46.

Brown, W. M., Yowell, C. A., Hoard, T. A., Vander Jagt, L. A., Hunsaker, L. M., Deck, R.E., *et al.* (2004). Comparative structural analysis and kinetic properties of lactate dehydrogenases from the four species of human malarial parasites. Biochemistry, 43**,** pp. 6219–6229.

Carneiro, I.A., Drakeley, C.J., Owusu-Agyei, S., Mmbando, B., Chandramohan, D. (2007). Haemoglobin and haematocrit: is the threefold conversion valid for assessing anaemia in malaria endemic settings? *Malaria Journal*, 6, P. 67.

Centers for Disease Control (C.D.C). (2002). Congenital malaria as a result of *Plasmodium malariae*-North Carolina, 2000.MMWR M orb Mortal wkly Rep, 51(8), pp.164-5.

Centers for Disease Control (C.D.C). (2006). Division of Parasitic Diseases National Center for Zoonotic, Vector-Borne, and Enteric Diseases (ZVED); nciddpdmalaria@cdc.gov

Centers for Disease Control (C.D.C). (2007). Division of Parasitic Diseases National Center for Zoonotic, Vector-Borne, and Enteric Diseases (ZVED).

Chalumeau, M., Holvoet, L., Cheron, G., Minodier, P., Foix-L'Helias, L., Ovetchkine, P., *et al.* (2006). Delay in diagnosis of imported *Plasmodium falciparum* malaria in children. *European Journal of Clinical Microbiology & Infectious Diseases*, 25, pp. 186–189.

Chandler, C.I., Chonya, S., Boniface, G., Juma, K., Reyburn, H., Whitty, C.J. (2008). The importance of context in malaria diagnosis and treatment decisions – a quantitative analysis of observed clinical encounters in Tanzania. *Tropical Medicine International Health*, 13, pp. 1131-1142.

Chandler, C.I., Jones, C., Boniface, G., Juma, K., Reyburn, H., Whitty, C.J. (2008). Guidelines and mindlines: why do clinical staff over-diagnose malaria in Tanzania? A qualitative study. *Malaria Journal*, 7, P.53.

Chandler, C.I., Mwangi, R., Mbakilwa, H., Olomi, R., Whitty, C.J., Reyburn, H. (2008). Malaria overdiagnosis: is patient pressure the problem? *Health Policy Plan*, 2, pp. 170-178.

Chandramohan, D., Jaffar, S. and **Greenwood, B. (2002).** Use of clinical features for diagnosing malaria. *Tropical Medicine International Health*, 7, pp. 45-52.

Chandy, C. John., Riedesel, A.M., Ng'wena, G.Magak., Kim. A., Hodges and Willis Akhwale. *et al*. (2009). Possible Interruption of Malaria Transmission, in highland Kenya. *Emerging Infectious Diseases (EID) Journal*, Vol. 15, No. 12.

Chandy, C.John., Koech, D.K., Sumba, P.O., Ouma, J.H. (1997). Risk of *Plasmodium falciparum* infection during a malaria epidemic in epidemic malaria-prone highland Kenya. Acta Tropica, volume 92, Issue: 1, pp. 55-61.

Cheesbrough, M. (1987). Medical laboratory Manual for Tropical countries. Butterworth- Heinemann Educational Low-Priced Books Scheme publishers. pp. 184-5.

Chilton, D., Malik, A.N., Armstrong, M., Kettelhut, M., Parker-Williams, J., Chiodini, P.L. (2006). Use of rapid diagnostic tests for diagnosis of malaria in the UK. *Journal of Clinical Pathology,* 59, pp. 862–866.

Chiodini, P. L., Bowers, K., Jorgensen, P., Barnwell, J.W., Grady, K.K., Luchavez, J .(2007). The heat stability of Plasmodium lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 101, pp. 331–337.

Chotivanich, K., Udomsangpetch, R., Dondorp, A., Williams, T., Angus, B., Simpson, *et al.* (2000).The mechanisms of parasite clearance after antimalarial treatment of Plasmodium falciparum malaria. Journal of Infections Diseases, 182, pp. 629-33.

Chotivanich, K., Silamu,t K., Day, N.P.J. (2006). Laboratory diagnosis of malaria infection-a short review of methods. *Aust Journal of Medical Science,* 27, pp. 11–15.

Clarke, S., Brooker, S., Njagi, J.K., Njau,E., Estambale,B., Muchiri,E and Magnussen, P. (2004). Malaria morbidity among school children living in two areas of contrasting transmission in western Kenya. *American Journal for Tropical Medicine and Hygiene,* 71(6), pp. 732–738.

Clendennen, T.E., Long,G.W., Baird, J.K. (1995). QBC® and Giemsa-stained thick blood films: diagnostic performance of laboratory technologists. *Transactions*

of the Royal Society of Tropical Medicine and Hygiene, 89 (2), pp.183-184. from: http://www.sciencedirect.com/science

Coleman, R. E., Maneechai, N., Rachaphaew, N., Kumpitak, C., Miller, R. S., Soyseng, V., *et al*. (2002). Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* in western Thailand. *American Journal for Tropical Medicine and Hygiene,* 67**,** pp.141–144.

Coleman, R. E., Sattabongkot, J., Promstaporm, S., Maneechai, N., Tippayachai, B., Kengluecha, A., *et al.* (2006). Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. *Malaria Journal,* 5, P.121.

Cooke, A.H., Chiodini, P.L., Doherty, T., Moody, A.H., Ries, J., Pinder, M., (1999). Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL) with microscopy for the detection of malaria parasites in human blood samples. *American Journal for Tropical Medicine and Hygiene,* 60, P.173– 6.

Cornet, M., Le Hesran, J-Y., Fievet, N., Cot, M., Personne, P., Gournoue, R., *et al.* (1998). Prevalence of and risk factors for anaemia in young children in southern Cameroon. *American Journal of Tropical Medicine and Hygiene,* 58, pp.606-11.

Cormack, D.S.F. (eds). (1982). The Researcher in Nursing. (2nd ed). London: Blackwell Scientific Publishers.

Craig, M.H., Snow, R.W., le Sueur, D. (1999). A climate-based distribution model of malaria transmission in sub-Saharan Africa. Parasitol Today, 15, pp.105–11.

Craig, M. H., Bredenkamp, B. L., Williams, C. H., Rossouw, E. J., Kelly, V. J., Kleinschmidt, I., *et al.* (2002). Field and laboratory comparative evaluation of

ten rapid malaria diagnostic tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96**,** pp. 258–265.

Crawley J. (2004). Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: From evidence to action. *American Journal of Tropical Medicine and Hygiene,* 71**,** pp. 25–34.

D'Acremont, V., Lengeler, C., Genton, B. (2007). Stop ambiguous messages on malaria diagnosis. *British Medical Journal,* 334**,** pp.489.

D'Acremont, V., Lengeler, C., Mshinda, H., Mtasiwa, D., Tanner, M. (2009). Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children withfever PLoSMed 6(1):e252. doi:10.1371/journal.pmed. 0050252.

Dal-Bianco, M.P., Köster, K.B., Kombila, U.D., Kun, J.F., Grobusch, M.P., Ngoma, G.M., *et al.* (2007). High prevalence of asymptomatic *Plasmodium falciparum* infection in Gabonese adults. *American Journal of Tropical Medicine and Hygiene,* 77**,** pp. 939–942.

DeMaeyer, E. and **Adels-Tegman, M. (1985).** The prevalence of anaemia in the world. World Health Statistics Quarterly, 38, pp.302-16.

Desai, M.R., Terlouw, D.J., Kwena, A.M., Phillips-Howard, P.A., Kariuki, S.K.,Wannermuehler, K.A., *et al.* (2005). Factors associated with haemoglobin concentrations in pre-school children in western Kenya: Cross-sectional studies. *American Journal of Tropical Medicine and Hygiene*, 72, pp.47-59.

Division of Malaria Control, 2011. Malaria in Kenya.

Doderer, C., Heschung, A., Guntz, P., Cazenave, J.P., Hansmann, Y., Senegas, A., *et al.* (2007). A new ELISA kit which uses a combination of *Plasmodium falciparum* extract and recombinant *Plasmodium vivax* antigens as an alternative to IFAT for detection of malaria antibodies. *Malaria Journal,* 6, P.19.

Dondorp, A. M., Desakorn, V., Pongtavornpinyo, W., Sahassananda, D., Silamut, D., Chotivanich, P. N., *et al*. (2005). Estimation of the total parasite biomass in acute *P.falciparum* malaria from plasma PfHRP2. PLoS Med. 2:Se204.

Drakeley, C.J., Carneiro, I., Reyburn, H., Malima, R., Lusingu, J.P., Cox, J., *et al.* (2005). Altitude-dependent and-independent variations in *Plasmodium falciparum* prevalence in northeastern Tanzania. *Journal for Infectious Diseases,* 191**,** pp.1589–1598.

Duffy, P and **Fried, M. (2005).** Malaria: New Diagnostics for an old problem. *American Journal of Tropical Medicine and Hygiene,* 73 (3), pp. 482-483.

Durand, F., Crassous,B., Fricker-Hidalgo ,H., Carpentier,F., Brion J.-P., Grillot ,R and H. Pelloux.(2005). Performance of the Now Malaria rapid diagnostic test with returned travellers: a 2-year retrospective study in a French teaching hospital. *Clinical Microbiology and Infection Journal*, Volume 11 Issue 11, pp. 903 – 907.

Durrheim, D. N., P. J. Becker, and K. Billinghurst. (1997). Diagnostic disagreement - the lessons learnt from malaria diagnosis in Mpumalanga. South African Medical Journal, 87, P.1016.

Eduardo Villamor., Maulidi, R., Fataki, Roger., L. Mbise and Wafaie W. Fawzi. (2009). Malaria parasitaemia in relation to HIV status and vitamin A supplementation among pre- school children.*Tropical Medicine & International Health,* Volume 8 Issue12, Journal compilation © Blackwell Publishing Ltd. Published Online: 27 Nov 2003.pp. 1051 –1061.

Egan, A., Crawley, J., Schellengerg, D., IPTi Consortium. (2005). Intermittent preventive treatment for malaria control in infants: moving towards evidence-based policy and public health action. *Tropical Medicine and International Health,* 10, pp. 815-7.

Ekvall, H., Premji, Z., Bjorkman, A. (1998). Chloroquine treatment for uncomplicated childhood malaria in an area with drug resistance: early treatment

failure aggravates anaemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 92, pp. 556-60.

Ekvall, H., Premji, Z., Bennet, S., Bjorkman, A.(2001). Haemoglobin concentration in a malaria holoendemic area is determined by cumulatve *Plasmodium falciparum* parasite densities. *American Journal of Tropical Medicine and Hygiene,* 64, pp.58-66.

Elizabeth, A. Juma., Charles, O. Obonyo., Willis, S. Akhwale and Bernhards, R. Ogutu. (2008). A randomized, open-label, comparative efficacy trial of artemether-lumefantrine suspension versus artemether-lumefantrine tablets for treatment of uncomplicated *Plasmodium falciparum* malaria in children in western Kenya *Malaria Journal*, 7, P.262.

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P.M., Shargie, E.B., Ejigsemahu, Y., *et al.* (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*, 5, P.118.

English, M., Esamai, F., Wasunna, A., Wamae, A., Snow, R.W., Peshu,**N. *et al.* (2004).** Assessment of inpatient paediatric care in first referral level hospitals in 13 districts in Kenya. *Lancet*, 363, pp.1948-1953.

English, M., Esamai, F., Will beunna, A., Were, F., Ogutu, A., Snow,R.W. *et al.* (2004). Delivery of paediatric care at the first referral level in Kenya. *The Lancet*, 364, pp.1622-1629.

English, M., Reyburn, H., Goodman, C., Snow, R.W. (2009). Abandoning presumptive antimalarial treatment for febrile children aged less than five years—A case of running before we can walk? *PLoS Med* 6(1): e1000015. doi: 10.1371/journal.pmed.1000015.

Erdman, L.K and **Kain, K.C. (2008).** Molecular diagnostic and surveillance tools for global malaria control. *Travel Medical Infectious Diseases*, 6, pp.82–99.

Ernst, K.C., Adoka, S.O., Kowuor, D.O., Wilson, M.L., John, C.C. (2006). Malaria hotspotareas in a epidemic malaria-prone highland Kenya site are consiste in epidemic and non-epidemic years and are associated with ecological factors. *Malaria Journal*, 5, P.78.

Fleming, A.F and **Werblinska, B. (1982).** Anaemia in childhood in the guinea savannah of Nigeria. *Annals of Tropical Paediatrics*, 2, pp.161-173.

Forney, J. R., C., Wongsrichanalai, A. J., Magill, L. G., Craig, J., Sirichaisinthop, C., T. Bautista., *et al.* (2003). Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect *Plasmodium falciparum* histidine-rich protein 2 and a *Plasmodium vivax*-specific antigen. *Journal for Clinical Microbiology*, 41, pp. 2358–2366.

Gasser, R. A., Magill A. J., Ruebush, T., Miller R. S., Sirichaisinthop, J., Forney, et al. (2005). Malaria diagnosis: performance of NOW ICT Malaria in a large scale field trial. Abstr. 54th Annu. Meet. *American Journal of Tropical Medicine and Hygiene*, abstr. 2338.

Gay, L., Mills, G. and **Peter Airasian. (2006).** Educational Research: Competencies for Analysis and Application, 8th Ed. Prenntice-Hall, ISBN 0-13-118534-9.

Geissler, P.W., Mwaniki, D.L., Thiong'o, F., Michaelsen, K.F., Friis, H. (1998). Geophagy, iron status and anaemia among primary school children in western Kenya. Tropical Medicine and International Health, 3, pp.529-34.

Genrich, G. L., Guarner, J., Paddock, C. D., Shieh, W. J., Greer, P. W., Barnwell, J. W. and Zaki, S. R. (2007). Fatal malaria infection in travelers: novel immunohistochemical assays for the detection of Plasmodium falciparum in tissues and implications for pathogenesis. *American Journal of Tropical Medicine and Hygiene*, 76, pp. 251–259.

Giha, H.A., A-Elbasit, I.E., A-Elgadir, T.M. (2005). Cerebral malaria is frequently associated with latent parasitemia among the semi-immune population of eastern Sudan. *Microbes Infections,* 7, pp. 1196 –203.

Githeko, A.K., Ayisi, J.M., Odada. P.K., Atieli. F.K., Ndenga, B. A., Githure, J.I., Yan, G. (2006). Topograph and malaria transmission heterogeneity in western Kenya highlands: prospects for focal vector control. *Malaria Journal*, 5**,** P.107.

Githeko A, Lindsay S, Confalonieri U, Partz J. (2000). Climate changes and vector borne diseases: a regional analysis. Bulletin of the World Health Organization, 78**,** pp. 1136–47.

Githeko, A and **Ndegwa, W. (2001).** Predicting malaria epidemics using climate data in Kenyan epidemic malaria-prone highlands: a tool for decision makers. Global Change and Human Health, 2**,** pp.54–63.

Goodman, C., Coleman, P., Mills, A. (2000). Improving malaria case management. *In*: Goodman,C, Coleman P, Mills A, eds. *Economic analysis of malaria control in sub-Saharan Africa*. Geneva, Global Forum for Health Research, pp.109–129.

Graham, S.M., Walsh, A.L., Molyneux, E.M., Phiri, A.J., Molyneux, M.E. (2000). Clinical presentation of non-typhoidal Salmonella bacteraemia in Malawian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94,310-4.

Greenberg, A.E., Nguyen-Dinh, P., Mann, J.M., Kabote, N., Colebunders, R.I., Francis, H. (1988). The association between malaria, blood transfusions and HIV seropositivity in a paediatric population in Kinshasa, Zaire; *Journal of the American Medical Association,* 259, pp.545-549.

Greenwood, B. (2002). The molecular epidemiology of malaria. *Tropical Medicine International Health,* 7**,** pp.1012–1021.

Greenwood, B.M., Greenwood, A.M., Bradley, A.K., Tulloch, S., Hayes, R., Oldfield, F.S. (1987). Deaths in infancy and early childhood in a well-vaccinated, rural, West African population. *Annals of Tropical Paediatrics,* 7, pp. 91–99.

Greenwood, B.M., Fidock, D.A., Kyle, D.E., Kappe, S.H., Alonso, P.L., Collins, F.H., et al. (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigations,* 118, pp.1266–76.

Guerrant , R.L., Walker, D.H., Waller, P.F (eds) and Krogstad, D.J. (1999). Tropical infectious diseases. Principles and Practice. Churchill Livingstone. P. 736.

Guerra, C.A., Gikandi, P.W., Tatem, A.J., Noor, A.M., Smith, D.L. (2008). The limits and intensity of Plasmodium falciparum transmission: Implications for malaria control and elimination worldwide.PLoS Medicine, 5:e38. doi: 10.1371/journal.pmed.0050038.

Guthmann, J.P., Ruiz, A., Priotto, G., Kiguli, J., Bonte, L., Legros, D. (2002). Validity, reliability and ease of use in the field of five rapid tests for the diagnosis of *Plasmodium falciparum* malaria in Uganda. Transactions of the Royal Society Tropical Medicine and Hygiene, 96**,** pp. 254 –7.

Hamer, D.H., Ndhlovu, M., Zurovac, D., Fox, M., Yeboah-Antwi, K., Chanda, P., Sipilinyambe, Hanscheid, T., Grobusch, M.P. (2002). How useful is PCR in the diagnosis of malaria? Trends Parasitol, 18, pp. 395-398.

Hamer, D. H., M. Ndhlovu, D., Zurovac, M., Fox, K., Yeboah-Antwi, P., Chanda, N. Sipilinyambe, J. L. Simon, and R. W. Snow. (2007). Improved diagnostic testing and malaria treatment practices in Zambia. JAMA, 297**,** 2227–2231.

Hanscheid, T. (1999). Diagnosis of malaria: a review of alternatives to conventional microscopy. Clinical and Laboratory Haematology, 21, pp. 235–245.

Hans, E.T., Watanabe, R., Sattabongkot, J., Khuntirat, B., Sirichaisinthop, J., Iriko, H., Jin, L., Takeo, S., Tsuboi, T. (2007). Detection of four *Plasmodium* species by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. Journal of Clinical Microbiology, 45, pp. 2521-2528.

Hanscheid, T., Melo-Cristino, J., Pinto, B.G. (2001). Automated detection of malaria pigment in white blood cells for the diagnosis of malaria in Portugal. *American Journal of Tropical Medicine and Hygiene,* 64, pp. 290-292.

Harvey, S.A., Jennings, L., Chinyama, M., Masaninga, F., Mulholland, K., Bell, D.R. (2008). Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training. *Malaria Journal*, 7, **P.160**.

Hawkes, M and **Kain, K.C. (2007).** Advance in malaria diagnosis. Expert Rev Anti Infect Ther, 5**,** pp. 1-11.

Hay, S.I., Cox, J., Rogers, D.J., Randolph, S,E., Stern, D.I., Shanks, G.D., *et al*. (2002). Climate change and the resurgence of malaria in the East African seasonally epidemic malaria-prone highlands.*Nature,* 415**,** pp. 905–9.

Hay, S.I., Simba, M., Busolo, M., Noor, A.M., Guyatt, H.L., Ochola, S.A., *et al.* (2002). Defining and detecting malaria epidemics in the highlands of western Kenya. Emerging Infectious Diseases, 8, pp. 555–62.

Hayward, R. E., D. J. Sullivan., and K. P. Day. (2000). *Plasmodium falciparum*: histidine-rich protein II is expressed during gametocyte development. Exp. Parasitology, 96**,** pp. 139–146.

Hedberg, K., Shffer, N., Davachi, F., Hightower,A., Lyamba, B., Paluku,K.M. (1993). *Plasmodium* malaria associated sweating in children at a large hospital in Zaire. *American Journal of Tropical Medicine and Hygiene*, 48(3), pp.365-71.

Holland, C.A and **Kiechle, F.L. (2005).** Point-of-care molecular diagnostic systems-past, present and future. *Current Opinion Journal of Microbiology*, 8, pp.504–509.

Hopkins, H., Bebell, L., Kambale, W., Dokomajilar, C., Rosenthal, P.J. (2008). Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *Journal of Infectious Diseases,* 197, pp.510–518.

Howard, R. J.S., Uni, M., Aikawa, S., B. Wellems, T. E., Rener, J. and Taylor, D. W. *et al*. (1986). Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes. *Journal for Cell Biology*, 103**,** pp.1269–1277.

Imwong, M., Pukrittayakamee, S., Pongtavornpinyo, W., Nakeesathit, S., Day, N.P., White, N.J. *et al*. (2008). Gene amplification of the multidrug resistance 1 gene of *Plasmodium vivax* isolates from Thailand, Laos, and Myanmar. *Antimicrobial Agents Chemotherapy*, 52**,** pp.2657-2659.

Ingrid, Van Den Broek., Olivia Hill., Fabiola Gordillo., Bibiana Angarita., Prudence Hamade., Helen Counihan and Jean-Paul Guthmann. (2006). Evaluation of three rapid tests for diagnosis of *P. falciparum* and *P. vivax*.malaria in Colombia. *American Journal of Tropical Medicine and Hygiene*, 75 (6), pp. 1209-1215.

Iqbal, J., A. Siddique, M. Jameel, and P. R. Hira. (2004). Persistent histidinerich protein 2, parasite lactate dehydrogenase, and panmalarial antigen. *Microbiology Journal*, 42, pp.4237–4241.

Iqbal, J., Sher, A., Rab, A. (2000). *Plasmodium falciparum* histidine-rich protein 2-based immunocapture diagnostic assay for malaria: cross-reactivity with rheumatoid factor. *Journal of Clinical Microbiology*, 38**,** pp.1184–1186.

Ishengoma, D.R., Rwegoshora, R.T., Mdira, K.Y., Kamugisha, M.L., Rønn, A.M., Magesa, S.M. *et al*. (2009). Health laboratories in the Tanga region of Tanzania: the quality of diagnostic services for malaria and other communicable diseases. *Annals of Tropical Medicine and Parasitology*, 103, pp.441-453.

Ishengoma, D.R., Derua, Y.A., Rwegoshora, R.T., Tenu, F., Massaga, J.J.,,Magesa, S.M. *et al*. (2010). The performance of health laboratories and the quality of malaria diagnosis in six districts of Tanzania. *Annals of Tropical Medicine and Parasitology*, 104**,** pp.123-135.

Jamshaid, I., Arif., Nabila, M., Mohammed, K., Ahmed, A. (2003). Performance of the optimal test for malaria diagnosis among suspected malaria patients at the rural health centers. The American Society of Tropical Medicine and Hygiene, 68(5), 2003, pp. 624-628.

Jelinek, T., L. Amsler, M. P. Grobusch, and H. D. Nothdurft. (1999). Self-use of rapid tests for malaria diagnosis by tourists. Lancet, 354**,** pp.1609.

Jelinek,T.,Grobusch,M.P., Schwenke,S., Steidl,S., Klein,E and T. Löscher. *et al.* (1999). Sensitivity and Specificity of Dipstick Tests for Rapid Diagnosis of Malaria in Nonimmune Travelers. *Journal of Clinical Microbiology,* Vol. 37, No. 3, pp. 721-723.

Joshi, H. (2003). Markers for population genetic analysis of human plasmodia species, *P. falciparum* and *P. vivax. Journal of.Vector-Borne Diseases*, 40, pp.78–83.

Kachur, S. P., Nicolas, E., Jean-Francois, F., Benitez, A., Ruebush II, T, K. and Nguyen-Dinh, P. *et al.* (1998). Prevalence of malaria parasitemia and accuracy of microscopic diagnosis in Haiti, *Rev. Panama Salud Publica*, 3, pp.35–39.

Kacey, C.E., Adoka, S.O., Kowuor, Dickens, O., Wilson, M.L., Chandy, C.John, (2006). Malaria hotspot areas in a epidemic malaria-prone highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. *Malaria Journal*; Volume: 5, Issue: 78, Publisher: *BioMedical Central Journal*, P.78.

Kahigwa, E., Schellenberg, D., Sanz, S., Aponte, J.J., Alonso, P., Menendez, C. *et al.* (2002). Risk factors for presentation to hospital with severe anaemia in Tanzanian children: a case-control study. *Tropical Medicine and International Health*,**7**, pp.823-30.

Kain, K.C., Harrington, M.A., Tennyson, S., Keystone, J.S. (1998). Imported malaria: prospective analysis of problems in diagnosis and management. *Clinical Infectious Diseases Journal,* 27, 142–149.

Karbwang, J. (1996). ParaSight®–F test for the detection of treatment failure in multi-drug-resistant *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90, pp.513–515.

Kassu, A and **Aseffa, A. (1999).** Laboratory services in health centres within Amhara region, north Ethiopia. *East African Medical Journal*, 76, pp. 239-242.

Kenya; Ministry of Health. (2008 ed.). National Guidelines for Diagnosis, Treatment and Prevention of Malaria for Health professionals in Kenya. Division of Malaria Control, Ministry of Health. **dcdc@health.go.ke**

Kenya National Bureau of Statistics. (2011): Population of local authorities

Kenya Soil Survey. (1986). Ministry of Agriculture, National Agricultural Laboratories. Exploratory Soil Survey Report No. E1:KSS, Nairobi.

Kim, S.H., Nam, M.H., Park, G.H., Han, E.T., Klein, T.A., Lim, C.S. *et al.* (2008). Evaluation of a rapid diagnostic test specific for *Plasmodium vivax*. *Tropical Medicine International Health,* 13**,** pp.1495–1500.

Kitchen, A. D., and **Chiodini, P. L. (2006).** Malaria and blood transfusion. Vox Sang. 90, pp.77–84.

Kitua, A.Y., Smith, T.A., Alonso, P.I., Urassa, H., Kimario, J., Tanner,M. *et al.* (1997). The roles of low-level P.falciparum parasitaemia and sweating among infants living in an area of intense and perennial transmission. *Tropical Medicine International Health,* 2(4), pp.325-33.

Kothari, C.R. (2nd ed.) (1990). Research Methodology. Wishwa Prakashan publishers pp.217-218.

Kyabayinze, D.J., Tibenderana, J.K., Odong, G.W., Rwakimari, J.B., Counihan, H., (2008). Operational accuracy and comparative persistent antigenicity of HRP2 rapid diagnostic tests for *Plasmodium falciparum* malaria in a hyperendemic region of Uganda. *Malaria Journal*, 7, P. 221.

Lackritz, E.M., Campell, C.C., Ruebush, T.K., Wakube, W., Steketee, R.W., Were, J.B.O. *et al.* (1992). Effect of blood transfusion on survival among children in a Kenyan hospital. Lancet, 340, pp. 524-28.

Laferi, H., Kandel, K., Pichler, H. (1997). False-positive dipstick test for malaria. *New England Journal of Medicine*, 337**,** pp.1635–1636.

Lee, N., Baker, J., Andrews, K. T., Gatton, M. L. Bell, D., Cheng, Q. and J.McCarthy. (2006). Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. *Journal of Clinical Microbiology*, 44, pp. 2773–2778.

Lee, N., J. Baker, D. Bell, J. McCarthy, and Q. Cheng. (2006). Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests. *Journal of Clinical Microbiology*, 44, pp. 4547–4549.

Lee, S.W., Jeon, K., Jeon, B.R., Park, I. (2008). Rapid diagnosis of vivax malaria by the SD Bioline Malaria Antigen test when thrombocytopenia is present. *Journal of Clinical Microbiology*, 46, pp. 939–942.

Leke, R. F., Djokam, R. R., Zhou, Y., Cadigan, T., Parra, M and Taylor, D.W. *et al*. (1999). Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria. *Journal of Clinical Microbiology*, 37**,** pp. 2992–2996.

Lemma, H.R., Desta, A., Fottrel, E., Barnabas, B., Bianchi, A. (2008). Impact of deployment of artemether lumefantrine (AL) in combination with rapid diagnostic tests (MRDT) at community level in Tigray region, Northern Ethiopia [abstract FC018]. Strengthening Health Systems and the Global Health Workforce. Geneva Forum: *Towards Global Access to Health*, pp. 25–28.

Lindblade, K.A., Eisele, T.P., Gimnig, J.E., Alaii, J.A., Odhiambo, F., ter Kuile, F.O., et al. (2004). Sustainability of reductions in malaria transmission and infant

mortality in western Kenya with use of insecticide-treated bednets: 4 to 6 years of follow-up. JAMA, 291, pp. 2571–80.

Lindsay, S.W. and **Martens, W.J.M. (1998).** Malaria in the African epidemic malaria-prone highlands: past, present and future. *Bulletin of the World Health Organization,* 76, pp.33–45.

Loevinsohn, M. (1994). Climate warming and increased malaria incidence in Rwanda. *In*: *The Lancet,* vol. 343, pp.714-718.

Lubell, Y., Reyburn, H., Mbakilwa, H., Mwangi, R., Chonya, K., et al. (2007). The cost-effectiveness of parasitologic diagnosis for malaria-suspected patients in an era of combination therapy. *American Journal of Tropical Medicine and Hygiene,* 77**,** pp.128–132.

Lubell, Y., Hopkins, H., Whitty, C.M.W., Sarah, G. S and Anne, M. (2008). An interactive model for the assessment of the economic costs and benefits of different rapid diagnostic tests for malaria. *Malaria Journal*, 7, 21doi:10.1186/1475-2875-7-21.

Luckner, D., Lell, B., Grave, B., Lehman, L.G., Schmidt-Ort, R.J., Matousek, P *et al*. (1998). No influence of socioeconomic factors on severe malarial anaemia, hyperparasitaemia or reinfection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 92, pp.478-81.

Lusingu, J.P., Vestergaard, L.S., Jones,C., Akida,J., Savaeli,Z.X., Kitua,A.Y., Theanda,T,G. et al. (2004). Malaria morbidity and immunity among residents of villages with different P.f transmission intensity in N.E Tanzania. *Malaria Journal*, 3, P.26.

Luxemburger, C., F. Nosten, D. E. Kyle, L. Kiricharoen, T., Chongsuphajaisiddhi and N. J. White. (1998). Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low

transmission. *Transactions of the Royal Society Tropical Medicine and Hygiene*, 92, pp. 45–49.

Mabeza, G.F., Biemba, G., Brennan, A.G., Moyo, V.M., Thuma, P.E., Gordeuk, V.R. (1998). The association of pallor with haemoglobin concentrations and mortality in severe malaria. *Annals of Tropical Medicine and Parasitology*, 92, pp. 663-9.

Macleod, J., Edwards, C and Boucher, I., (Eds.) (1987). Davidson's Principles and Practice of Medicine. Longman Group. UK Ltd. P.772.

Maeno, Y., Nakazawa, S., Daole, D., Yamamoto, N., Van, Hanh, T., *et al*. (2008). A dried blood sample on filter paper is suitable for detecting *Plasmodium falciparum* gametocytes by reverse transcription polymerase chain reaction. *Acta Tropica Journal,* 107, pp. 121–7.

Makani, J., Matuja, W., Liyombo, E., Snow, R.W., Marsh, K., Warrell, D.A. (2003). Admission diagnosis of cerebral malaria in adults in an endemic area of Tanzania implications and clinical description. *Q Journal Medicine.*, 96, pp.355–362.

Malakooti, M.A., Biomndo, K., Shanks, G.D. (1998). Re-emergence of epidemic malaria in the epidemic malaria-prone highlands of western Kenya. *Emerging Infectious Diseases*, 4, pp.671–6.

Makler, M. T., and **Hinrichs, D. J. (1993).** Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. *American Journal of Tropical Medicine and Hygiene*, 48, pp. 205–210.

Makler, M. T., Piper, R. C. and Milhous, W. K. (1998). Lactate dehydrogenase and the diagnosis of malaria. *Parasitology Today*, 14, pp. 376–377.

Makler, M.T., Palmer, C.J. and Ager, A.L. (1998). A review of practical techniques for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology*, 92, pp.419–433.

Manjurano, A., Okell, L., Lukindo, T., Reyburn, H., Riley, M and Drakeley, C. *et al.* (2011). Association of sub-microscopic malaria parasite carriage with transmission intensity in north-eastern Tanzania, *Malaria Journal,* 10, 370 doi:10.1186/1475-2875-10-370.

Map data ©2011Google.

Marx, A., Pewsner, D., Egger, M., Nuesch, R., Bucher, H.C. (2005). Meta-analysis: Accuracy of rapid tests for malaria in travelers returning from endemic areas. Annas of International Medicine, 142, pp. 836–846.

Masika, P.M., Semarundu, W.J., Urassa, R., Mosha, J., Chandramohan, D., Gosling, R.D. (2006). Over-diagnosis of malaria is not a lost cause. *Malaria Journal*, 5, P.120.

Mason, D. P., Kawamoto, F., Lin, F., Laoboonchai, A and Wongsrichanalai., C. (2002). A comparison of two rapid field immunochromatographic tests toexpert microscopy in the diagnosis of malaria. *Acta Tropica*, 82, Issue 1, pp. 51–59.

Mayxay, M., Pukrittayakamee, S., Chotivanich, K., Looareesuwan, S., White, N.J. (2001). Persistence of *Plasmodium falciparum* HRP-2 in successfully treated acute falciparum malaria. *Transactions of the Royal Society Tropical Medicine and Hygiene*, 95**,** pp.179–82.

Mboera, L.E., Fanello, C.I., Malima, R.C. (2006). Comparison of the Paracheck-Pf test with microscopy, for the confirmation of *Plasmodium falciparum* malaria in Tanzania. *Annals of Tropical Medicine and Parasitology*,100, pp.115–22.

McElroy, P.D., Ter Kuile, F.O., Lal, A.A., Bloland, P.B., Oloo, Nahlen, B.L, et al. (2000). Effect of *Plasmodium parasitaemia* density on haemoglobin concentrations among full normal birth weight children in western Kenya, IV. The Asembo Bay cohort project. *American Journal of Tropical Medicine and Hygiene,* 62, pp. 504-12.

McFarland, W., Mvere, D., Shandera, W., Reingold, A. (1997). Epidemiology and prevention of transfusion-associated human immunodeficiency virus transmission in sub-Saharan Africa. *Vox Sanguinis*,72, pp. 85-92.

McGregor, L.A., Williams, K., Billewicz, W., Thompson, A. (1966). Haemoglobin concentration and anaemia in young West African (Gambian) children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 60, pp. 650-667.

McKenzie, F. E., Sirichaisinthop, J., Miller, R. S., Gasser, R. A. Jr., and Wongsrichanalai, C. (2003). Dependence of malaria detection and species diagnosis by microscopy on parasite density. *American Journal of Tropical Medicine and Hygiene*, 69, pp. 372–376.

McMichael, A.J. (1997). Integrated assessement of potential health impact of global environmental change: prospects and limitations. Environmental modeling and assessment, vol., 9, P. 35.

McMorrow, M.L., Masanja, M.I., Abdulla, S.M., Kahigwa, E., Kachur, S.P. (2008). Challenges in routine implementation and quality control of rapid diagnostic tests for malaria-Rufiji District, Tanzania. *American Journal of Tropical Medicine and Hygiene,* 79, pp. 385–390.

Mendoza, N.M, García, M., Cortés, L.J., Vela, C., Ospina, O.L., Burgos, J.D., et al. (2007). Evaluation of two rapid diagnostic tests, NOW ICT Malaria Pf/Pv and OptiMAL, for diagnosis of malaria. *Biomedica*, 27(4), pp. 571-80.

Menegon, M., Severini, C., Sannella, A., Paglia, M.G., Sangare, D., Abdel-Wahab, A., et al. (2000). Genotyping of *Plasmodium falciparum* gametocytes by reverse transcriptase polymerase chain reaction. *Molecular Biochemistry and Parasitology*, 111**,** pp. 153–61.

Menendez, C., Fleming, A.F., Alonso, P.L. (2000). Malaria-related sweating. *Parasitology Today,* 16 (11), pp. 469-76.

Menge, D.M., Ernst, K.C., Vulule, J.M., Zimmerman, P.A., Guo, H., John, C.C. (2008). Microscopy underestimates the frequency of *Plasmodium falciparum*

infection insymptomatic individuals in a low transmission highland area. *American Journal of Tropical Medicine and Hygiene*, 79, pp. 173–177.

Mens, P., Spieker, N., Omar, S., Heijnen, M., Schallig, 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 Journal*, 12, 238–44.

Mens, P.F., Schoone, G.J., Kager, P.A., Schallig, H.D., (2006). Detection and identification of human *Plasmodium* species with real time quantitative nucleic acid sequence based amplification. *Malaria Journal*, 5, P.80.

Mens, P.F., van Amerongen, A., Sawa, P., Kager, P.A., Schallig, H.D. (2008). Molecular diagnosis of malaria in the field: development of a novel 1-step nucleic acid lateral flow immunoassay for the detection of all 4 human *Plasmodium spp*. and its evaluation inMbita, Kenya. *Diagnosis and Microbiology of Infectious Diseases,* 61, pp. 421-427.

Mharakurwa, S., Manyame, B., Shiff, C.J. (1997). Trial of the ParaSight-F test for malaria diagnosis in the primary health care system, Zimbabwe. *Tropical Medicine International Health Journal*, 2, pp. 544 –50.

Miller, R. L., S. Ikram, G. J., Armelagos, R.. Walker, W., Carrigan, B.M. and Maret, S. M. *et al.* (1994). Diagnosis of *Plasmodium falciparum* infections in mummies using the rapid manual ParaSight-F test. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88, pp. 31–32.

Miller, R. S., McDaniel, P. and Wongsrichanalai, C. (2001). Following the course of malaria treatment by detecting parasite lactate dehydrogenase enzyme. *British Journal of Haematology*, 113, pp. 558–559.

Milne, L. M., Kyi, M. S. Chiodini, P. L. and Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal of Clinical Pathology*, 47, pp.740–742.

Ministry of Health Guidelines for malaria epidemic preparedness and control in Kenya. Nairobi: The Ministry, Government of Kenya. (1999).

Ministry of Health (Kenya) National Malaria Strategy. (2001-2010). Nairobi: The MinistryGovernment of Kenya; 2001.

Mlambo, G., Vasquez, Y., LeBlanc, R., Sullivan, D., Kumar, N. (2008). A filter paper method for the detection of *Plasmodium falciparum* gametocytes by reverse transcription polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, 78, pp.114-116.

Mockenhaupt, F.P., Bedu-Addo, G., von Gaertner, C. (2006). Detection and clinical manifestation of placental malaria in southern Ghana. *Malaria Journal*, 5, P.119.

Moerman, F., Lengeler, C., Chimumbwa, J. (2003). The contribution of health- care services to a sound and sustainable malaria-control policy. *Lancet Infectious Diseases Journal*, 3, pp. 99 –102.

Molyneux, M. and **R. Fox. (1993).** Diagnosis and treatment of malaria in Britain. *British. Medical Journal*, 306, pp.1175-1180.

Molyneux, M. E and **Koram, K.A. (2007).** "When is 'Malaria' Malaria? The Different Burdens of malaria Infection, Malaria Disease and Malaria-like Illnesses *American Journal of Tropical Medicine and Hygiene*, 77 (6-supl), pp. 1-5.

Moody, A., Hunt-Cooke, A., Gabbett, E., Chiodini, P. (2000). Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London.*British Journal of Haematology*, 109, pp. 891– 4.

Moody, A. (2002). Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Rev. Joournal*, **15**, pp. 66–78.

Moody, A. H. and **Chiodini, P. L. (2002).** Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. British Journal of Biomedical Science, 59, pp.228–231.

Moore, A., Herrera, G., Nyamongo, J., Lackritz, E., Granade, T., Nahlen, B., Oloo, A., Opondo, G., Muga, R., Janssen, R, (2002). Estimated risk of HIV transmission by blood transfusion in Kenya. *Lancet*, 358, pp. 657-60.

Morassin, B., Fabre, R., Berry, A., Magnaval, J.F. (2002). One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. *American Journal of Tropical Medicine and Hygiene*, 66, pp.503–508.

Msellem, M.I., Martensson, A., Rotllant, G., Bhattarai, A., Ali, A., Bjørkman., *et al.* (2009). Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar:a crossover validation study. *PLoS Med*, 6: e1000070.

Muller, H.D., Abeku, T.A., Okia, M., Rapuoda, B and Cox, J. (2009). Costs of early detection systems for epidemic malaria in epidemic malaria-prone highland areas of Kenya and Uganda. Malaria Journal, 8, P.17.

Mueller, I., Betuela, I., Ginny, M., Reeder, J. C. and **Genton. B. (2007).** The sensitivity of the OptiMAL rapid diagnostic test to the presence of *Plasmodium falciparum* gametocytes compromises its ability to monitor treatment outcomes in an area of Papua New Guinea in which malaria is endemic. *Journal for Clinical Microbiology*, 45, pp. 627–630.

Mulenga, M., Malunga, P., Bennet, S., Thuma, P.E., Fielding, K., Greenwood, B.M. (2005). Factors associated with severe anaemia in Zambian children admitted with *Plasmodium falciparum* malarial anaemia. *Annals of Tropical Paediatrics*, 25, pp. 87-90.

Mugenda, O.M and **Mugenda, A.G. (2003).** Research Methods: Quantitative and Qualitative Approaches: Nairobi: ACTS Press.

Murphy, S.C and **Breman, J.G. (2001).** Gaps in the childhood malaria burden in Africa: Cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *American Journal of Tropical Medicine and Hygiene*, 64 (Suppl 1–2), pp. 57–67.

Murray, C. K., Mody, R. M., Dooley, D. P., Horvath, D. L. L., Moran, K. A. and Muntz, R. W. *et al.* (2006). The remote diagnosis of malaria using telemedicine or e-mailed images. Mil. Medicine, 171**,** pp.1167–1171.

Murray, C.K., Gasser, R.A, Jr., Magill, A.J and Scott Millers, R. (2008). Update on Rapid Diagnostic Testing for Malaria. Clinical Microbiology Reviews, Vol. 21, pp. 97–110.

Mwangi, T.W., Mohammed, M., Dayo, H., Snow, R.W., Marsh, K. (2005). Clinical features for malaria diagnosis lack utility among people of different age groups. *Tropical Medicine and International Health*, 10**,** pp.530–536.

Mwanzi, H.A. (1977). *A history of the Kipsigis*, Nairobi, East African Literature Bureau.

Nabil, S.A and **Qader, S.S. (2009).** Is global warming likely to cause an increased incidence of malaria? Libyan Journal of Medicine, **4** (1), pp.18-22.

Najera, J.A. (1989). *Malaria and the work of WHO Bullettin of the World Health Organization,* 67, pp.229–243.

Nandi District Development Plan. (1997-2001). Office of the Vice President and Ministry of Planning and National Development *Government Printer.*

Nandi District Strategic Plan. (2005-2010) for Implementation of the National Population Policy for Sustainable Development. National Coordination Agency for Population and Development Ministry of Planning and National Development Publishers. Email:ncpd@skyweb.co.ke

Nankabirwa,J.,Zurovac,D.,Njogu,J.N.,Rwakimari, J.B., Snow.,R.W.,Tibenderana. J.K., et al. (2009). Malaria misdiagnosis in Uganda--implications for policy change. *Malaria Journal*, 8, P.66.

National Malaria Strategy (2001-2010). The Republic of Kenya, Ministry of Health.

Ndyomugyenyi, R., Magnussen, P., Clarke, S. (2007). Diagnosis and treatment of malaria in Peripheral health facilities in Uganda. Findings from an area of low transmission in south-west Uganda. *Malaria Journal*, 6, 39 doi 10; 1186/1475-2875-6-39.

Ngasala, B., Mubi, M., Gustafsson, L.L., Tomson, G., Premji, Z., Bjorkman, A., *et al.* (2008). Impact of training in clinical and microscopy diagnosis of childhood malaria on antimalarial drug prescription and health outcome at primary healthcare level in Tanzania: a randomized controlled trial. *Malaria Journal*, 7, P.199.

Ngindu, A.M., Kabiru, E.W., Mbaabu, D.A.N., Odero, W.O.O., Siongok,T.K. (1989). Outbreak of epidemic malaria in Uasin Gishu District in 1988**.** Proceedings of the 10th Annual Medical Scientific Conference, Nairobi, Kenya. KEMRI/ KETRI.

Nicoll, A. (2000). Integrated management of childhood illness in resource-poor countries: an initiative from the World Health Organization. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94, pp. 9–11.

Njama-Meya, D., Clark, T.D., Nzarubara, B., Staedke, S., Kamya, M.R., *et al.* (2007). Treatment of malaria restricted to laboratory-confirmed cases: A prospective cohort study in Ugandan children. *Malaria Journal*, 6, P. 7.

Njuguna, J., Muita, J and Mundia, G. (2009). Malaria morbidity and temperature variation in a low risk Kenyan district: a case of overdiagnosis? International Journal of Biometeorology Springer Berlin / Heidelberg publishers; Volume 53, Number 3, pp. 299-304.

Nussenblatt, V. and **Semba, R.D. (2002).** Micronutrient malnutrition and the pathogenesis of malarial anaemia. *Acta Tropica*, 82, pp.321-337.

Nyame, A. K., Kawar, Z. S. and **Cummings, R. D. (2004).** Antigenic glycans in parasitic infections: implications for vaccines and diagnostics. Arch. Biochem. Biophys. Journal, 426, pp.182–200.

Nyamongo,I.K. (2002). Healthcare switching behaviour of malaria patients in a Kenyan rural community. Social Science Medical Journal, 54 (3), pp. 377-386.

Obonyo, C.O., Ochieng, F., Taylor, W.R., Olliaro, P., ter Kuile, F., Oloo, A.J., *et al.* (2003). Artesunate plus sulfadoxine-pyrimethamine for uncomplicated malaria in Kenyan children: a randomized, double-blind, placebo-controlled trial. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 97(5), pp.585-91.

Obonyo, C.O. (2006). Malaria, anaemia and antimalarial drug resistance in African children. Thesis, University of Utrecht, Utrecht, The Netherlands. ISBN-10: 90-393-4348-9; ISBN-13: 978-90-393-4348-7. P.29-31.

Ochola, L.B., Vounatsou, P., Smith, T., Mabaso, M.L., Newton, C.R. (2006). The reliability of diagnostic techniques in diagnosis and management of malaria in absence of a gold standard. *Lancet Infectious Diseases Journal*, pp.582–588.

O'Dempsey, T.J., McArdle, T.E., Laurence, B.E., Lamont, A.C., Todd, J.E., Greenwood, B.M. (1993). Overlap in the clinical features of pneumonia and malaria in African children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, pp. 662-5.

Oduola, A.M., Omitowoju, G.O., Sowunmi, A. (1997). *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. Exp Parasitology, 87, pp. 283–9.

Ogutu, B.R., Smoak, B.L., Nduati, R.W., Mbori-Ngacha, D.A., Mwathe, F., Shanks, G.D. (2000). The efficacy of pyrimethamine-sulfadoxine (Fansidar) in the treatment of uncomplicated *Plasmodium falciparum* malaria in Kenyan children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94(1), pp. 83-4.

Oh, J.S., Kim, J.S., Lee, C.H., Feldmann, H., Briese,T., Lipkin, W.I., *et al.* (2007). Panmicrobial oligonucleotide array for diagnosis of infectious diseases. *Emerging Infectious Diseases*, 13, pp.73-81.

Ohrt, C. Purnomo., Sutamihardia, M.A., Tang, D., Kain, K.C. (2002). Impact of microscopy error on estimates of protective efficacy in malaria prevention trials. *Journal of Infectious Diseases,* 186, pp.540–546.

Ohrt, C., P. Obare., C. Adhiambo., W. P.O'Meara., J. S. Odera., and B. Ogutu. *et al.* (2007). Establishing a malaria diagnostics centre of excellence in Kisumu, Kenya. *Malaria Journal*, 6, pp.79.

Okell, L.C., Ghani, A.C., Lyons, E., Drakeley, C.J. (2009). Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. *Journal for Infectious Diseases*, 200, pp. 1509–1517.

Okiro, E.A., Hay, S.I., Gikandi, P.W., Sharif, S.K., Noor, A.M., Peshu, N., et al. (2007). The decline in paediatric malaria admissions on the coast of Kenya. *Malaria Journal*, 6, P. 151.

Oloo, A.J., Vulule, J.M., Koech, D.K. (1996). Some emerging issues on the malaria problem in Kenya. *East African Medical Journal*,73, pp. 50–3.

Olivier ,B., Sandrine H. , Yves Se´Cardin, Jean, P. Coulaud and **Jacques ,L. (2000).** *Use of the parasight-f diagnostic test for imported malaria in a Travel clinic. American Journal of Tropical Medicine and Hygiene*, 63(1, 2), pp. 76–79.

Omumbo, J., Ouma, J., Rapuoda, B., Craig, M.H., Le Sueur, D., Snow, R.W. (1998). Mapping malaria transmission intensity using geographical information systems (GIS): an example from Kenya. *Annals of Tropical Medicine and Parasitology*, 92, pp. 7–21.

Ong'echa, J.M., Keller, C.C., Were, T.O.C., Otieno, R.O., Landis-Lewis Z. (2006). Parasitaemia, anaemia and malarial anaemia in infants and young children in a rural holoendemic *Plasmodium falciparum* transmission area. *American Journal of Tropical Medicine and Hygiene*, 74, pp. 376-85.

Otieno, R.O., Ouma, C., Ongecha, J.M., Keller, C.C., Were, T., Waindi, E.N. *et al.* (2006). Increased severe anaemia in HIV-exposed and HIV-positive infants and children during acute malaria. AIDS 20, 275-80.

Owusu-Agyei, S., Fryauff, D.J., Binka, F.N., Nkrumah, F.K., Utz, G.C., Hoffman, S., *et al.* (2002). Characteristics of severe anaemia and its association with malaria in young children of the Kassena-Nankana District of Northern Ghana. *American Journal of Tropical Medicine and Hygiene*, 673, pp.71-77.

Padial, M.M., Subirats, M., Puente, S., Crespo, S., Palacios, G., Baquero, M., *et al.* (2005). Sensitivity of laser light depolarization analysis for detection of malaria in blood samples. *Journal of Medical Microbiology*, 54, pp. 449-452.

Park, T.S., Kim, J.H., Kang, C.I., Lee, B.H., Son, H.C., Kim, H.H. *et al.* (2006). Diagnostic usefulness of SD malaria antigen and antibody kits for differential diagnosis of vivax Malaria in patients with fever of unknown origin. *Korean Journal of Laboratory Medicine*, 26, pp. 241–245.

Park, G.H. (2008). Evaluation of a malaria antibody enzyme immunoassay for use in blood screening. *Mem Inst Oswaldo Cruz Journal*, 103, pp.75–78.

Patarakul, K. (2008). Role of DNA microarray in infectious diseases. *Chula Medical Journal*, 52, pp. 147-153.

Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin World Health Organization*, 66, pp. 621–628.

Phillips, R.E and **Pasvol, G. (1992)**. Anaemia of *Plasmodium falciparum malaria. Balliere's Clinical Haematology,* 5, pp.315-329.

Perkins, B.A., Zucker, J.R., Otieno, J., Olargo, C., Gove, S., Campbell, C.C. et al. (1997). Evaluation of an algorithm for integrated management of childhood illness in an area of Kenya with high malaria transmission. *Bulletin of the World Health Organization,* 75, pp.33–42.

Perkins, M.D., Bell, D.R. (2008). Working without a blindfold: the critical role of diagnostics in malaria control. *Malaria Journal*, **7** Suppl 1: S5.

Peters, R. P., Zijlstra, E. E., Schijffelen, M. J.., Kublin, G., Molyneux, M. E. and D. K. Lewis., *et al.* (2004). A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management. *Tropical Medicine and International Health Journal*, 9, pp**.**928–934.

Petti, C.A., Polage, C.R., Quinn, T.C., Ronald, A.R., Sande, M.A. (2006). Laboratory medicine in Africa: a barrier to effective health care. *Clinical Infectious Diseases Journal*, 42, pp. 377-382.

Piper, R., Lebras, J., Wentworth, L. (1999). Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). *American Journal of Tropical Medicine and Hygiene,* 60, pp.109 –18.

Polage, C.R., Bedu-Addo, G., Owusu-Ofori, A., Zurcher, E., Hale, D., Petti, C.A., *et al.* (2006). Laboratory use in Ghana: physician perception and practice. *American Journal of Tropical Medicine and Hygiene,* 75**,** pp.526-531.

Poon, L.L., Wong, B.W., Ma, E.H., Chan, K.H., Looareesuwan, S., Peiris, J.S. (2006). Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clinical Chemistry Journal*, 52, pp.303-306.

Pornsilapatip, J., Namsiripongpun, V., Wilde, H., Hanvanich, M., Chutivongse, S. (1990). Detection of Plasmodia in acridine orange stained capillary tubes (the QBC system). *Southeast Asian Journal of Tropical Medicine and Public Health,* 21, pp.534–540.

Premji,Z., Hamisi, Y., Shiff, C., Minjas, J., Lubega,P., Makwaya, C. (1995). Sweating and *Plasmodium falciparum* infections among young children in an holoendemic area, Bagamoyo, Tanzania. Acta Tropica, 59 (1), pp. 55-64.

Premji, Z., Minjas, J.N., Shiff, C.J. (1999). Laboratory diagnosis of malaria by village healthworkers using the rapid manual ParaSight™–F test. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88, pp. 418.

Price, R.N., Simpson, J.A., Nosten, F., Luxemburger, C., Hkirjaroen, L., Ter Kuile, F., et al. (2001). Factors contributing to anaemia after uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene,* 65**,** pp.614-32.

Puta, C. and **Manyando, C. (1997).** Enhanced gametocyte production in Fansidar-treated *Plasmodium falciparum* malaria patients: implications for malaria transmission control programmes. *Tropical Medicine and International Health Journal*, 2, pp. 227–229.

Rafael, M.E., Taylor, T., Magill, A., Lim, Y.W., Girosi, F., Allan, R. (2006). Reducing the burden of childhood malaria in Africa: the role of improved. *Nature Journal*, 444 (Suppl 1), pp. 39–48.

Ratsimbasoa, A., Rakotonirina, H., Barnadas, L., Jahevitra, M., Andriantsoanirina, V., Ménard, D., et al. (2008). Accuracy and reliability of malaria diagnostic techniques for guiding febrile outpatient treatment in malaria-endemic countries. *American Journal of Tropical Medicine and Hygiene,* 78, pp. 217–221.

Redd, S.C., Wirima, J.J., Steketee, R.W, (1994). Risk factors for anaemia in young children in rural Malawi. *American Journal of Tropical Medicine and Hygiene,* 51, pp.170-174.

Reinhartz, M.C., Ambroise-Thomas, P., Cavallo-Serra, R., Meylan, C., Gautier, R., (1978). Malaria at delivery in A. Helvetica Acta-supplementum, 41, 65-84.

Reinhartz, A., Alajem, S., Samson, A., and Herzberg, M. (1993). A novel rapid hybridization technique: paper chromatography hybridization assay (PACHA). Gene, 136, pp. 221-22.

Reyburn, H., Mbatia, R., Drakeley, C., Olomi, R., Greenwood, B.M., Whitty, C.J.M., *et al.* (2004). Overdiagnosis of malaria in patients with severe febrile

illness in Tanzania: a prospective study. *British Medical Journal*, 329, pp.1212–1215.

Reyburn, H., Mbakilwa, H., Mwangi, R., Mwerinde, O., Drakeley, C., and **Whitty, C. J., *et al.* (2007).** Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *British Medical Journal*, 334, P.403.

Richter, J., Gobels, K., Muller-Stover, I., Hoppenheit, B. and Haussinger, D. (2004). Co-reactivity of plasmodial histidine-rich protein 2 and aldolase on a combined immuno-chromographic-malaria dipstick (ICT) as a potential semi-quantitative marker of high *Plasmodium falciparum* parasitaemia. *Parasitology, Res. Journal*, 94, pp. 384–385.

Rock, E. P., Marsh, K., Saul, A. J., Taylor, D. W., Maloy, W. L. and Howard, R. J., *et al.* (1987). Comparative analysis of the *Plasmodium falciparum* histidine-rich proteins HRP-I, HRP-II and HRP-III in malaria parasites of diverse origin. *Parasitology Journal*, 95, pp. 209–227.

Ronald, L.A., Kenny,S.L., Akoto,A.O., Boakye,I., Barnish,G., Donnelly, M.J., *et al.* (2006). Malaria and sweating among children in two communities of Kumas Ghana: a cross-sectional study. *Malaria Journal*, 5, P.105.

Seed, C. R., Kitchen, A. and Davis. T. M. (2005). The current status and potential role of laboratory testing to prevent transfusion-transmitted malaria. Transfusion. Medicine.Rev. Journal, 19, pp.229–240.

Shanks, G.D., Biomndo, K., Hay, S.I., Snow, R.W. (2000). Changing patterns of clinical malaria since 1965 among a tea estate population located in the Kenyan epidemic malaria-prone highlands. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94**,** pp. 253–5.

Shanks, G.D., Biomndo, K., Maguire, J. (2004). Travel as a risk factor for malaria requiring hospitalization on a epidemic malaria-prone highland tea plantation in western Kenya. *Journal of.Travel Medicine*, 11 (6), pp.354-7.

She, R.C., Rawlins, M.L., Mohl, R., Perkins, S.L., Hill, H.R., Litwin, C.M. (2007). Comparison of immunofluorescence antibody testing and two enzyme immunoassays in the serologic diagnosis of malaria. *Journal of.Travel Medicine*, 14, pp. 105–111.

Shekalaghe, S. A., Bousema, J. T., Kunei, K. K. Mosha, W. Sauerwein, R. W. and Drakeley, C. J. *et al.* (2007). Submicroscopic *Plasmodium falciparum* gametocyte carriage is common in an area of low and seasonal transmission in Tanzania.*Tropical Medicine International Health Journal*, 12, pp. 547–553.

Schellenberg, J. R., Smith, T., Alonso, P. L. and Hayes, R. J. (1994). What is clinical malaria? Finding case definitions for field research in highly endemic areas. *Parasitology Today*, 10, pp. 439–442.

Schellenberg, D., Armstrong-Schellenberg, J.R.M., Mushi, A., Mgalula, L., Mbuya, C., Victoria, C.G., *et al.* (2003). The silent burden of anaemia in Tanzanian children: a community-based study. *Bulletin of the World Health Organization,* 81, pp. 581-90.

Scholl, P.F., Kongkasuriyachai, D., Demirev, P.A., Lin, J.S., Sullivan, D.J. Jr., Kumar, N. *et al.* (2004). Rapid detection of malaria infection *in vivo* by laser desorption mass spectrometry. *American Journal of Tropical Medicine and Hygiene*, 71, pp. 546-551.

Shiff, C. J., Premji, Z. and Minjas, J. N. (1993). The rapid manual ParaSight®–F test. A new diagnostic tool for *Plasmodium falciparum* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, pp. 646–648.

Shiff, C.J., Minjas, J., Premji, Z. (1994). The ParaSight®–F test: A simple rapid manual dipstick test to detect *Plasmodium falciparum* infection. *Parasitology Today*, 10, pp. 494–495.

Shillcutt, S., Morel, C., Goodman, C., Coleman, P., Bell, D., *et al.* (2008). Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy. *Bulletin of the World Health Organization*, 86, 101–110.

Singer, L. M., Newman, R. D., Diarra, A., R. Sawadogo., J. W. Barnwell., and M. E. Parise., *et al.* (2004). Evaluation of a malaria rapid diagnostic test for assessing the burden of malaria during pregnancy. *American Journal of Tropical Medicine and Hygiene*, 70, pp. 481–485.

Singh, N and **Shukla, M.M. (2002).** Short report: Field evaluation of posttreatment sensitivity for monitoring parasite clearance of *Plasmodium falciparum* malaria by use of the Determine Malaria pf test in central India. *American Journal of Tropical Medicine and Hygiene*, 66, pp. 314–6.

Sing, A. E., Rauch, A., Roggenkamp, I., Autenrieth, B. and **Heesemann, J. (2000).** Evaluation of the ICT malaria Pf test for rapid post-mortem diagnosis of *Plasmodium falciparum* malaria in corpses examined for forensic reasons. *International Journal of Legal Medicine*, 113, pp. 251–252.

Slutsker, L., Taylor, T.T., Wirima, J.J., Steketee, R.W. (1994). In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88, pp. 548-51.

Smith, T., Schellenberg, J.A., Hayes, R. (1994). Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Medicine*, 13, pp. 2345-58.

Snounou, G., and **White, N. J. (2004).** The co-existence of Plasmodium: sidelights from *P. falciparum* and *P. vivax* malaria in Thailand. *Trends in Parasitology*, 20, pp.333–339.

Snounou, G., Viriyakosol, S., Zhu, X.P., do Rosario, V.E.,Thaithong, S., Brown., K.,N., *et al.* (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Molecular Biochemistry and Parasitology*, 61, 315-320.

Snow, R.W., Omumbo, J.A., Lowe, B., Molyneux, C.S., Obiero, J.O., Palmer, A., *et al.*, (1997). Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet*, 349, pp. 1650–4.

Snow, R.W., Gouws, E., Omumbo, J., Tanser, F.C., le Sueur, D. and **Ouma, J., (1998).** Models to predict the intensity of *Plasmodium falciparum* transmission: applications to the burden of disease in Kenya. *In: East African Medical Journal*, vol. 71 (1), pp. 2-8.

Snow, R.W. (1999). Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of the World Health Organization*, 77(8), pp.624-40.

Snow, R.W., Trape, J-F., Marsh, K., (2001). The past, present and future of childhood mortality in Africa.*Trends in Parasitology*, 17, pp. 593–7.

Some, E.S. (1994). Effects and control of epidemic malaria-prone highland epidemic malaria in Uasin Gishu district, Kenya. *East African Medical Journal*, 71(1), pp. 2-8.

Sokhna, C. S., Trape, J. F. and **Robert. V. (2001).** Gametocytaemia in Senegalese children with uncomplicated falciparum malaria treated with chloroquine, amodiaquine or sulfadoxine -pyrimethamine. *Parasite*, 8, pp. 243–250.

Sowunmi, A. and **Akindele, J.A. (1993).** Presumptive diagnosis of malaria in infants in an endemic area. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, P.422.

Stephens, J.K. (1999). A comparison of three malaria diagnostic tests, under field conditions in north-west Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 30, pp. 625–630.

Suh, I. B., Choi, H. K., Lee, S. W., Won, Y. D., Cho, M., and **Lim, C. S., *et al.* (2003).** Reactivity of sera from cases of Plasmodium vivax malaria towards three recombinant antigens based on the surface proteins of the parasite. *Annals of Tropical Medicine & Parasitology*, 97, pp. 481–487.

Susi, B., Whitman, T., Blazes, D. L., Burgess, T. H., Martin, G. J. and **Freilich, D. (2005).** Rapid diagnostic test for *Plasmodium falciparum* in 32 Marines medically evacuated from Liberia with a febrile illness. *Annals of Tropical Medicine & Parasitology*, 142, pp. 476–477.

Swan, H., Sloan, L., Muyombwe, A., Wilairatana, P., Looareesuwan, S., Rosenblatt, J., *et al.* (2005). Evaluation of a real-time polymerase chain reaction assay for the diagnosis of malaria in patients from Thailand. *American Journal of Tropical Medicine and Hygiene*, 73, pp. 850-854.

Swarthout, T.D., Counihan, H., Senga, R.K., van den Broek, I. (2007). Paracheck-Pf(R) accuracy and recently treated *Plasmodium falciparum* infections: is there a risk of over-diagnosis? *Malaria Journal*, 6, P.58.

Swill beh, M. (ed.) (2002). *Hutchison's Clinical methods.* (21st ed).W.B Saunders Publishers. P. 141.

Tagbor, H., Jane Bruce., Edmund Browne., Brian Greenwood. (2008). *Malaria infection in pregnancy.* Therapeutics and Clinical Risk Management. Dove Medical Press Ltd. 4(3), pp. 631–636.

Tan, T. M. C., Nelson, J. S., Ng, H. C., Ting, R. C. Y. and **Kara, U. A. K. (1997).** Direct PCR amplification and sequence analysis of extrachromosomal *Plasmodium* DNA from dried blood spots. *Acta Tropical Journal*, 68**,** pp.105-114.

Tarimo, D.S., Lwihula, G.K., Minjas, J.N., Bygbjerg, I.C. (2000). Mothers' perceptions and knowledge on childhood malaria in the holendemic Kibaha district, Tanzania: implications for malaria control and the IMCI strategy. *Tropical Medicine International Health Journal*, 5, pp. 179-184.

Tarimo, D.S., Minjas, J.N., Bygbjerg, I.C. (2001). Malaria diagnosis and treatment under the strategy of the integrated management of children illness (IMCI): relevance of laboratory support from the rapid immunochromatographic tests of ICT malaria *P.f/P.v* and OptiMAL. *Annals of Tropical Medicine & Parasitology,* 95, pp. 437–444.

Tavrow, P., Knebel, E., Cogswell, L. (2000). Using quality design to improve malaria rapid diagnostic tests in Malawi. Bethesda, MD, Quality Assurance Project.*Operational ResearchResults,*1 (4). www.wpro.who.int/MRDT/link11.asp

Taylor, T.E., Fu, W.J., Carr, R.A., Fosiko, N.G., Liomba N.G., Molyneux , M.E., *et al.* (2004). Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med.*10: 143–145.The Malaria Working Party of the General Haematology Task Force of the British Committee for Standards in Haematology. The laboratory diagnosis of malaria. *Clinical and Laboratory Haematology Journal,* 19**,** pp. 165–170.

The Malaria Working Party of the General Haematology Task Force of the British Committee for Standards in Haematology, (1997).

Tjitra, E. (1999). Field evaluation of the ICT *P.f/P.v* immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with a presumptive clinical diagnosis of malaria in Eastern Indonesia. *Journal of Clinical Microbiology*, 37, pp. 2412–2417.

Tjitra, E., Suprianto, S., McBroom, J., Currie, B. J. and **Anstey, N. M. (2001).** Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia

and results in false-positive diagnoses of *Plasmodium vivax* in convalescence. *Journal of Clinical Microbiology*, 39, pp. 1025–1031.

Tjitra, E., Suprianto, S., Dyer, M.E., Currie, B.J., Anstey, N.M. (2001). Detection of histidine rich protein 2 and panmalarial ICT Malaria Pf/Pv test antigens after chloroquine treatment of uncomplicated *falciparum* malaria does not reliably predict treatment outcome in eastern Indonesia. *American Journal of Tropical Medicine and Hygiene*, 65, pp. 593– 8.

Trachsler, M., Schlagenhauf, P. and **Steffen, R. (1999).** Feasibility of a rapid dipstick antigen-capture assay for self-testing of travellers' malaria. *Tropical Medicine International Health Journal*, 4, pp.442–447.

Trape, J.F., Pison, G., Preziosi, M.P., Desgres du Lo, A., Delaunay, V., Samb, B., *et al.*, (1998). Impact of chloroquine resistance on malaria mortality. Comptes REnus Academy of Science III, 321**,** pp. 689-97.

Trape, J., Pison, G., Spiegel, A., Enel, C and **Christophe Rogier. (2002).** Combating malaria in Africa. *Trends in Parasitology Journal:* Volume 18, Issue 5, pp. 224-230.

Uasin Gishu District Development Plan (2002-2008). Effective Management for Sustainable Growth and Poverty Reduction. Government Printer, Nairobi.

Uasin Gishu District Strategic Plan (2005-2010). Implementation of the National Population Policy for Sustainable Development**.**

Uguen, C. (1995). ParaSight®–F rapid manual diagnostic test of *Plasmodium falciparum* infection. *Bulletin of the World Health Organization,* 73, pp. 643–649.

United States Agency for International Developmen-USAID. (2008). Current information on rapid diagnostic tests. The HealthTech IV program, Cooperative Agreement # GPH-A- 00-01-00005-00.

Van Eijk, A.M., Ayisi, J.G., Otieno, J.A., Kager, P.A., Steketee, R.W., Nahlen, B.I. *et al.* (2002). Malaria and human immunodeficiency virus infection as risk factors for infants in Kisumu, western Kenya. *American Journal of Tropical Medicine and Hygiene,* 67, pp. 44-53.

Verhoeff, F.H., Brabin, B.J., Masache, P., Kachale, B., Kazembe, P., Van der Kaay, H.J., *et al*. (1997). Parasitological and Haematological responses to treatment of *Plasmodium falciparum* malaria with sulfadoxine-pyrimethamine in Southern Malawi. *Annals of Tropical Medicine and Parasitology,* 91, pp. 133-40.

Verhoeff, H., West, C.E., Veenemans, J., Beguin, Y., Kok, F.J. (2002). Stunting may determine the severity of malaria-associated anaemia in African children. *Paediatrics* 110: e48

Véronique , D., Paul, B., Norman, B., Anne, M., Amagana, D and **Andre, R., *et al.* (2000).** What does a single determination of malaria parasite density mean? A longitudinal survey in Mali. *I*n: *The Journal of Tropical Medicine & International Health,* vol.5 (6), pp. 404.

Vo, T.K., Bigot, P., Gazin, P., Huynh, D.C., Fumoux, F., Parzy, D., *et al.* (2007). Evaluation of a real-time PCR assay for malaria diagnosis in patients from Vietnam and in returned travelers. *Transactions of the Royal Society of Tropical Medicine,* 101, pp. 422–428.

Von Schenck, H., Falkensson, M., Lundberg, B. (1986). Evaluation of 'Hemocue,' a new device for determining hemoglobin. *Clinical Chemistry*, 32, pp. 526-9.

Warhurst, D.C and **Williams, J.E. (1996).** Laboratory diagnosis of malaria. *Journal of Clinical Pathology,* 49, pp.533–538.

Wongchotigul, V., Suwanna, N., Krudsood, S., Nakayama, S., Kojima, S., Looareesuwan, S., *et al.* (2004). The use of flow cytometry as a diagnostic test for malaria parasites. *Southeast Asian Journal of Tropical Medicine and Public Health*, 35, pp.552-559.

World Health Organization (WHO, 1968). Nutritional anaemias. Report of a WHO Scientific Group. Geneva, World Health Organization, 1968 (WHO Technical Report Series, No. 405).

World Health Organization. (WHO, 1996). WHO information consultation on recent advances in diagnostic techniques and vaccines for malaria: a rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. *Bulletin of the World Health Organization,* 74, pp. 47–54.

World Health Organization (WHO, 1998). The use of artemisin and its derivatives as antimalarial drugs: a report of a joint CTD/DMP/TDR Informal Consultation. World Health Organization, Geneva, 1998 WHO/MAL/98. 1086.

World Health Organization. (WHO, 1999). "New perspectives: malaria diagnosis," Report of a Joint WHO/USAID Informal Consultation, World Health Organization, Geneva, October, 1999, Switzerland.

World Health Organization. (WHO, 2000). New perspectives. Malaria. Diagnosis. Report of a joint WHO/USAID informal consultation, 25–27 October 1999. WHO, Geneva, Switzerland.

World Health Organization (WHO, 2003). Guidelines for the diagnosis and treatment of malaria in the African Region. AFR/MAL/03.01.2003. World Health Organization, Regional Office for Africa, Brazaville, Congo.

World Health Organization (WHO, 2004). Informal consultation on laboratory methods for quality assurance of malaria rapid diagnostic tests: meeting report 20–22 July,2004. Manila, Regional Office for the Western Pacific, 2004 ((WP) MVP/ICP/MVP/1.4/001.E).

World Health Organization: Geneva; (WHO, 2005). Technical consultation to review the role of the parasitological diagnosis to support malaria disease management:

focus on the use of MRDT in areas of high transmission deploying ACT treatments.

World Health Organization (WHO, 2006). The use of malaria rapid diagnostic tests manual, second edition, 2006; Geneva,Switzerland.

World Health Organization (WHO, 2006). The role of laboratory diagnosis to support malaria disease management: focus on the use of rapid diagnostic tests in areas of high transmission.

World Health Organization (WHO, 2006). Towards quality testing of malaria rapid diagnostic tests: evidence and methods. WHO, Western Pacific Region, Manila, Philippines.

World Health Organization (WHO, 2006): Guideline for the treatment of malaria.WHO/HTM/MAL/2006.1108.

World Health Organization (WHO, 2008). Impact of long-lasting insecticidal-treated nets (LLINs) and artemisinin-based combination therapies (ACTs) measured using surveillance data, in four African countries. WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

World Health Organization. (WHO, 2008). World Malaria Report. WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

World Health Organization. (WHO, 2008). Partnership Roll Back Malaria. Global malaria action plan: Geneva: World Health Organization.

Weber, M.W., Mulholland, E.K., Jaffar, S., Troedsson, H., Gove, S., Greenwood, B.M., *et al.* (1997). Evaluation of an algorithm for the integrated management of childhood illness in an area with seasonal malaria in the Gambia. *Bulletin of the World Health Organization,* 75, pp. 25–32.

Yahya, A.D., Deus, R.S.I., Rwehumbiza, T. R., Julius, J. M., Leonard, E.G.M., Stephen, M. M., *et al.* (2011). Users' and health service providers' perception on quality of laboratory malaria diagnosis in Tanzania. *Malaria Journal* 10, P.78.

Zell, R. (2004). Global climate change and the emergence/re-emergence of infectious diseases. *International Journal of Medial Microbiology*, 293, pp. 16-26.

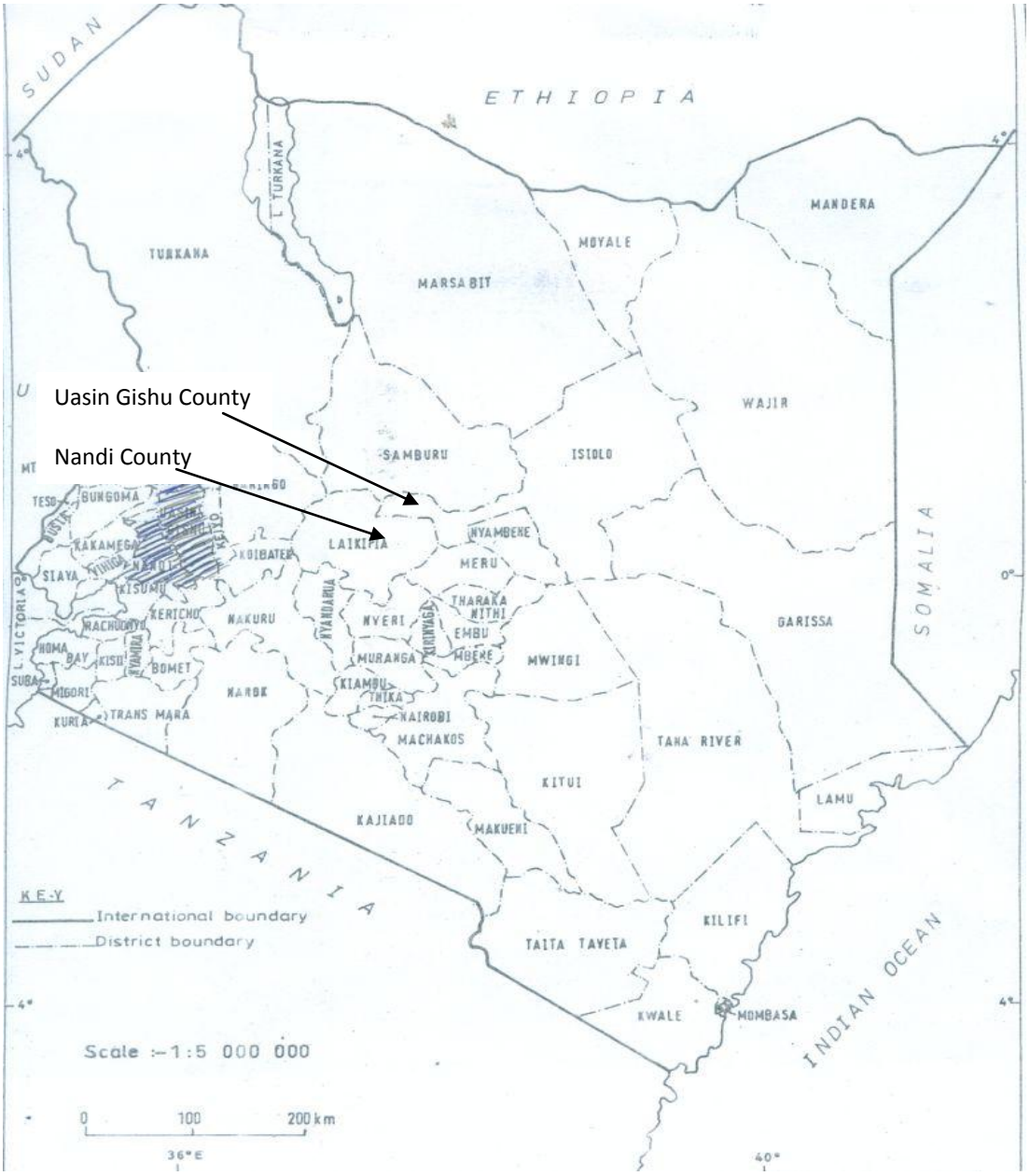
Zerpa, N., Pabón, R., Wide, A., Capaldo, J., Baker, M., Noya, O., *et al.* (2008). Evaluation of the OptiMAL test for diagnosis of malaria in Venezuela.. *Invest Clin. Journal*, 49, pp. 93–101.

Zucker, J.R., Lackritz, E.M., Ruebush, T.K., Hightower, A.W., Adungosi, J.E., Were, J.B.O., *et al.* (1996). Childhood mortality during and after hospitalization in western Kenya: effect of malaria treatment regimens. *American Journal of Tropical Medicine and Hygiene*, 59, pp. 808-12.

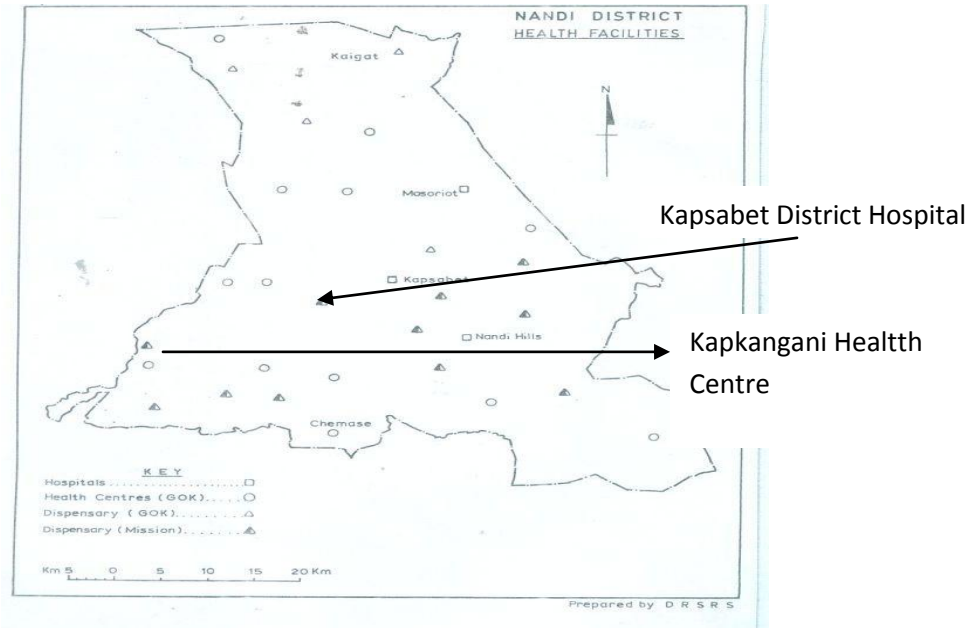
Zurovac, D., Njogu, J., Akhwale, W., Hamer, D.H., Larson, B.A., Snow, R.W. (2008). Effects of revised diagnostic recommendations on malaria treatment practices across age groups in Kenya. *Tropical Medicine International Health Journal*, 13, 784-787.

Zurovac, D., Midia, B., Ochola, S.A., English, M., Snow, R.W. (2006). Microscopy and outpatient malaria case management among older children and adults in Kenya. *Tropical Medicine International Health Journal*, 11, pp. 432-440.

APPENDICES

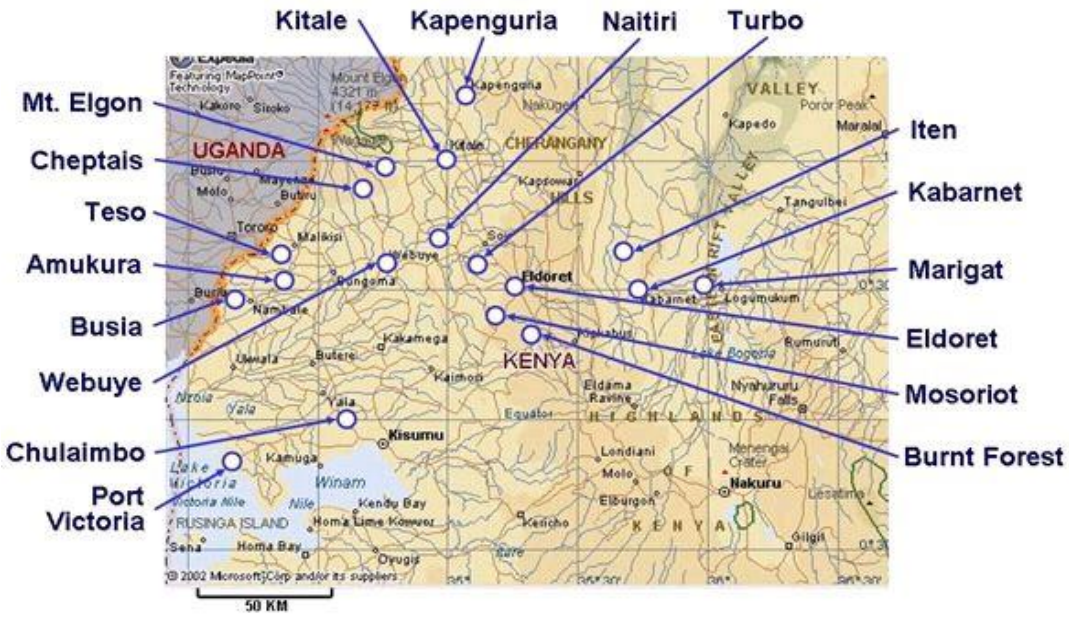


Appendix 1: Figure 3.1: Geographical Position of Nandi and Uasin Gishu Counties (Source: Uasin Gishu District Strategic Plan 2005-2010; Nandi District Development Plan 1997-2001).



Appendix 2: **Figure 3.2: Approximate Geographical Location of Main Study Area (In Nandi County)**

*(**Source**: Nandi District Development Plan, 1997-2001).*



Appendix 3: **Figure 3.3: Approximate Locations of Health Instutions where MRDT Questionnaires were distributed.** ***(Source: Map data ©2011Google).***

Appendix 4: **Figure 3.4: Approximate Geographical Location of Health Facilities in Study Area (Uasin Gishu County).**

(Source: *Uasin Gishu District Development Plan (2002-2008).*

Appendix 5: **Table 4.10: Commonest Malaria Clinical Features across Age groups of affected children <12yrs**

Clinical Feature	Age Distribution (% affected out of total population of Children studied)			**Total number and % population studied; (n=132)**	**P-values**
	0-60months (0-5years)	61-120months (5.1-10 years)	121-144months (10.1-12 years)		
Fever	47 (35.6%)	47 (35.6%)	28 (21.2%)	122 (92.4%)	P <0.05
Anaemia	45 (34.1%)	44 (33.3%)	24 (18.2%)	113 (85.6%	P <0.05
Sweating	48 (36.4%)	37 (28.0%)	22 (16.7%)	107 (81.1%)	P <0.05
Chills	30 (22.7%)	38 (28.8%)	24 (18.2%)	92 (69.7%)	P <0.05
Vomiting	37 (20.0%)	34 (25.8%)	18 (13.6%)	89 (67.4%)	P <0.05
Headache	22 (16.7%)	40 (30.3%)	24 (18.2%)	86 (65.2%)	P <0.05
Nausea	20 (15.2%)	20 (15.2%)	22 (16.7%)	62 (48.0%)	P <0.05
Diarrhoea	23 (17.4%)	24 (18.2%)	11 (8.3%)	58 (43.9%)	P >0.05
Some Dehydration	27 (20.5%)	20 (15.2%)	6 (4.5%)	53 (40.2%)	P>0.05
Convulsions	21 (15.9%)	10 (7.6%)	6 (4.5%)	37 (28.03%)	P >0.05
Severe Dehydration	9 (6.8%)	10 (7.6%)	11 (8.3%)	30 (22.7%)	P>0.05
Jaundice	11 (8.3%)	9 (6.8%)	9 (6.8%)	29 (22.0%)	P >0.05
Myalgia	5 \(3.8%)	5 (3.8%)	16 (12.1%)	26 (19.7%)	P>0.05
Backache	5 (3.8%)	5 (3.8%)	16 (12.1%)	26 (19.7%)	P >0.05
Splenomegaly	21 (15.9%)	11 (8.3%)	4 (3.0%)	23 (17.4%)	P>0.05
Joint pains	1 (0.8%)	6 (4.5%)	14 (10.6%)	21 (15.9%)	P >0.05
Hepatomegaly	7 (5.3%)	7 (5.3%)	1 (0.8%)	15 (11.4%)	P>0.05

Appendix 6A: **Table 4.14: Prevalence of five Commonest Complaints during 2005-2010, among children attending Turbo Health Centre, Uasin Gishu County in western Kenya highlands**

Morbidity Statistics for Five Commonest Complaints at Turbo Health Centre										
Year (Total population)	**D1**	**Prev. (%)**	**D2**	**Prev. (%)**	**D3**	**Prev. (%)**	**D4**	**Prev. (%)**	**D5**	**Prev. (%)**
2005 (n=3734)	Mal.	32.1	URTI	17.5	Skin	11.0	Assaults	5.7	Pneum.	4.6
2006 (n=3424)	Mal.	44.0	URTI	18.8	Skin	9.3	Arthritis	5.9	Diarrh.	3.5
2007 (n=3249)	Mal.	41.7	URTI	21.3	Skin	13.7	Assaults	11.3	Diarrh.	7.2
2008 (n=1984)	Mal.	39.0	URTI	13.1	Assaults	13.5	Skin	10.5	Diarrh.	5.7
2009 (n=997)	Mal.	45.1	URTI	10.9	Chn/pox	6.9	Diarr.	3.9	Skin	3.0
2010 (n=1085)	Mal.	48.8	URTI	10.9	Assaults	10.8	Typhoid	9.7	Skin	5.2

Key to Table 4.14: D1, D2, D3, D4, D5=Prevalence of various diseases (D) in sequence (D1to D5); Mal= Malaria; URTI=Upper respiratory tract infection; Chn/pox= Chicken pox; Diarrh. =Diarrhoea; Skin= Skin infections; Pneum.=Pneumonia.N.B: Prevalence figures were for rainy seasons(April-June) of each year. N.B: ***Total populations (n) attended in Turbo Health Centre for respective years during rainy season (April-June) were: n=3734, 3424, 3249, 1984, 997 and 1085 for the years: 2005-2010, respectively.***

Appendix 6B: Table 4.15: Prevalence of five Commonest Complaints during 2005-2010, among children attending Moi University (Main Kesses Campus Clinic), Uasin Gishu County in western Kenya highlands

Morbidity Statistics for Five Commonest Complaints at Moi Varsity (Main Campus)										
Year (Total population)	**D1**	**Prev. (%)**	**D2**	**Prev. (%)**	**D3**	**Prev. (%)**	**D4**	**Prev. (%)**	**D5**	**Prev. (%)**
2007 (n=5467)	URTI	26.0	Mal.	19.4	Skin	10.4	Accid.	6.6	Arthritis	4.0
2008 (n=5837)	URTI	29.7	Mal.	27.7	Arthritis	6.4	Skin	4.5	Accid.	1.9
2009 (n=4960)	URTI	25.3	Mal.	31.0	Arthritis	5.8	Skin	4.1	Accid.	3.8
2010 (n=4780)	URTI	28.9	Mal.	21.8	Skin	8.2	Arthritis	7.6	Accid.	3.5

Key to Table 4.15:D1, D2, D3, D4, D5=Prevalence of various diseases (D) in sequence (D1 toD5); Mal= Malaria; URTI=Upper respiratory tract infection; Accid.=Accidents/Assaults; Skin= Skin infections. ***N.B: Total populations (n) attended in Main Kesses campus (MU) Health Centre for respective years during rainy season (April-June) were: 5467, 5837, 4960 and 4780, respectively for the period between 2007 and 2010.***

Appendix 6C: Table 4.16: Prevalence of five Commonest Complaints during 2007-2010, among children attending Moi University (Medical School Campus Clinic), Uasin Gishu County in western Kenya Highlands

Morbidity Statistics for Five Commonest Complaints at Moi University (Med. School) Clinic										
Year (Total population)	**D1**	**Prev. (%)**	**D2**	**Prev. (%)**	**D3**	**Prev. (%)**	**D4**	**Prev. (%)**	**D5**	**Prev. (%)**
2007 (n=2619)	URTI	29.3	Mal.	12.5	D.Caries	6.6	Eye/inf.	5.5	Diarrh.	5.0
2008 (n=3791)	URTI	21.2	Mal.	14.7	D.Caries	6.3	Eye/inf.	3.7	Diarrh.	3.4
2009 (n=3246)	URTI	29.9	Mal.	12.4	Skin	6.4	D.Caries	5.1	Diarrh.	5.0
2010 (n=1450)	URTI	25.5	Mal.	16.4	Skin	14.3	Eye/inf.	6.3	Diarrh.	3.5

Key to Table 4.16:D1, D2, D3, D4, D5=Prevalence of various diseases (D) in sequence (D1 toD5); Mal= Malaria; URTI=Upper respiratory tract infection; Diarrh. =Diarrhoea; Skin= Skin infections; Eye/inf.=Eye infections; D.caries=Dental Caries. ***N.B: Total populations (n) attended in Town (Medical School) Health Centre for respective years during rainy season (April-June) were: 2619, 3791, 3246 and 1450 for the years: 2007-2010, respectively.***

Appendix 6D: Table 4.17: Prevalence of five Commonest Complaints during 2005-2010, among children attending University of Eldoret Clinic, Uasin Gishu County in western Kenya highlands

Morbidity statistics for Five Commonest Complaints at Chepkoilel University Clinic										
Year (Total population)	**D1**	**Prev. (%)**	**D2**	**Prev. (%)**	**D3**	**Prev. (%)**	**D4**	**Prev. (%)**	**D5**	**Prev. (%)**
2007 (n=3903)	URTI	37.7	Mal.	36.9	Skin	4.7	Arthritis	2.9	PUD	2.2
2008 (n=2615)	URTI	43.5	Mal.	41.2	STI	16.8	Diarrh.	15.8	I/Worms	15.3
2009 (n=3417)	URTI	25.6	Mal.	10.1	Skin	5.1	STI	1.8	Eye/inf.	1.4
2010 (n=1708)	URTI	27.1	Mal.	23.2	Skin	12.1	Diarrh.	1.6	Arthritis	1.4

Key to Table 4.17: ***D1, D2, D3, D4, D5=Prevalence of various diseases (D) in sequence (D1 toD5); Mal= Malaria; URTI=Upper respiratory tract infection; STI=Sexually Transmitted Infections; Diarrh.=Diarrhoea; Skin= Skin infections; PUD= Peptic Ulcer Disease; Intestinal worms. N.B: Total populations (n) attended in Chepkoilel University College Clinic during (May-July) were: 3903, 2615, 3417 and 1708 for the years: 2007-2010, respectively.***

Appendix 6E: Table 4.18: Prevalence of five Commonest Complaints during 2005-2010, among children attending Kapkangani Health Centre, Nandi County in western Kenya highlands

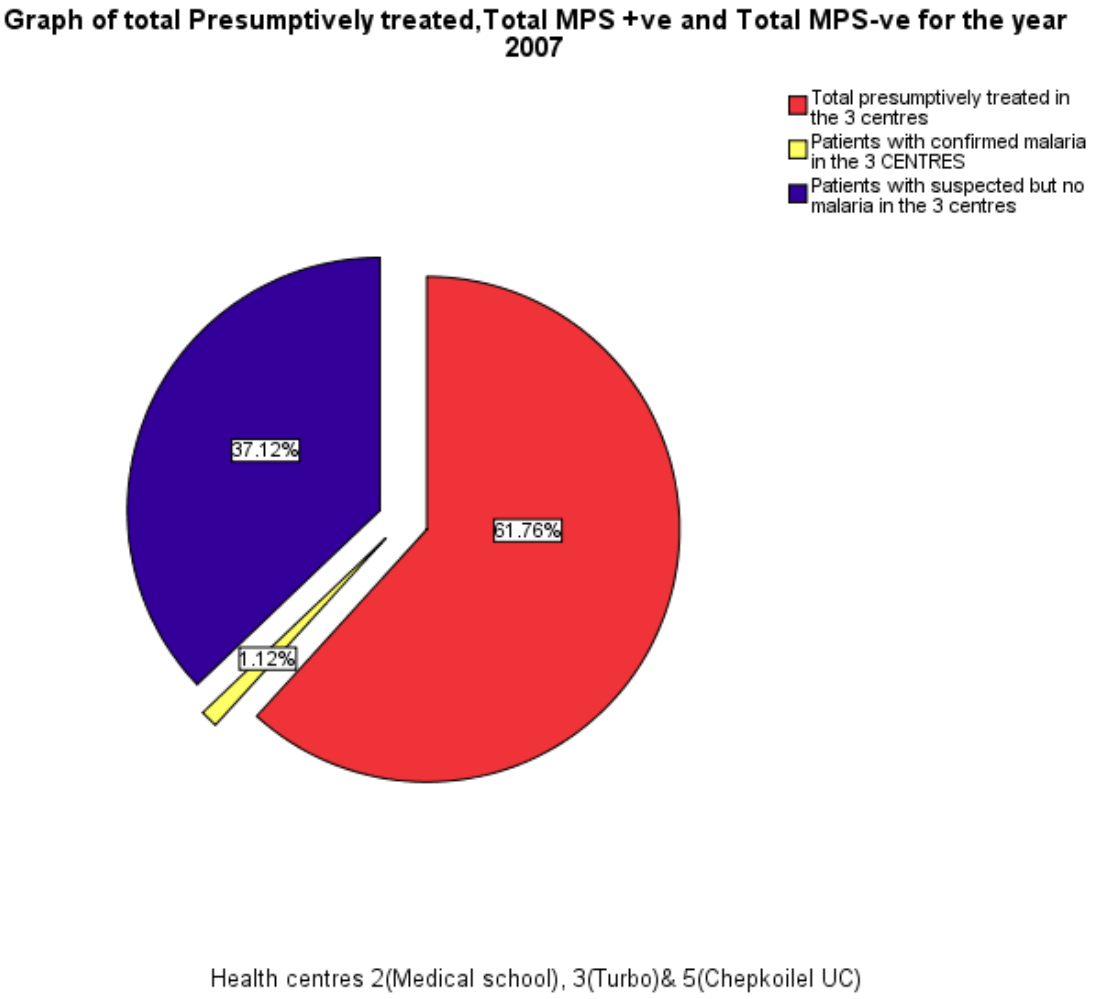
Year	D1	Prev. (%)	D2	Prev. (%)	D3	Prev. (%)	D4	Prev. (%)	D5	Prev. (%)
2005 (n=2890)	Mal.	21.4	URTI	27.4	Diarrh.	20.5	I/Worms	17.0	Pneum.	13.7
2006 (n=2837)	Mal.	34.6	URTI	21.4	Diarrh.	15.1	I/Worms	14.5	Pneum.	14.4
2007 (n=3303)	Mal.	28.0	URTI	20.9	Diarrh.	17.0	I/Worms	15.2	Pneum.	10.7
2008 (n=3328)	Mal.	30.2	URTI	27.7	Diarrh.	15.8	I/Worms	14.8	Pneum.	11.5
2009 (n=3741)	Mal.	32.3	URTI	27.3	Diarrh	18.5	I/Worms	11.0	Pneum.	10.8
2010 (n=3179)	Mal.	31.3	URTI	26.5	Diarrh.	18.3	I/Worms	12.4	Pneum.	11.6

Key to Table 4.18:*D1, D2, D3, D4, D5=Prevalence of various diseases (D) in sequence (D1 toD5); Mal= Malaria; URTI=Upper respiratory tract infection; Diarrh.=Diarrhoea; I/Worms=Intestinal worms; Pneum.=Pneumonia.* ***N.B: Total populations (n) attended in Health Centre for respective years during rainy season (April-June) were: 2890, 2837, 3303, 3328, 3741 and 3179 for the years: 2005-2010, respectively.***

Appendix 6F: **Table 4.19: Comparison of Presumptively treated, Suspected Malaria and Confirmed Malaria across Rural health centres in Western Kenya highland Region**

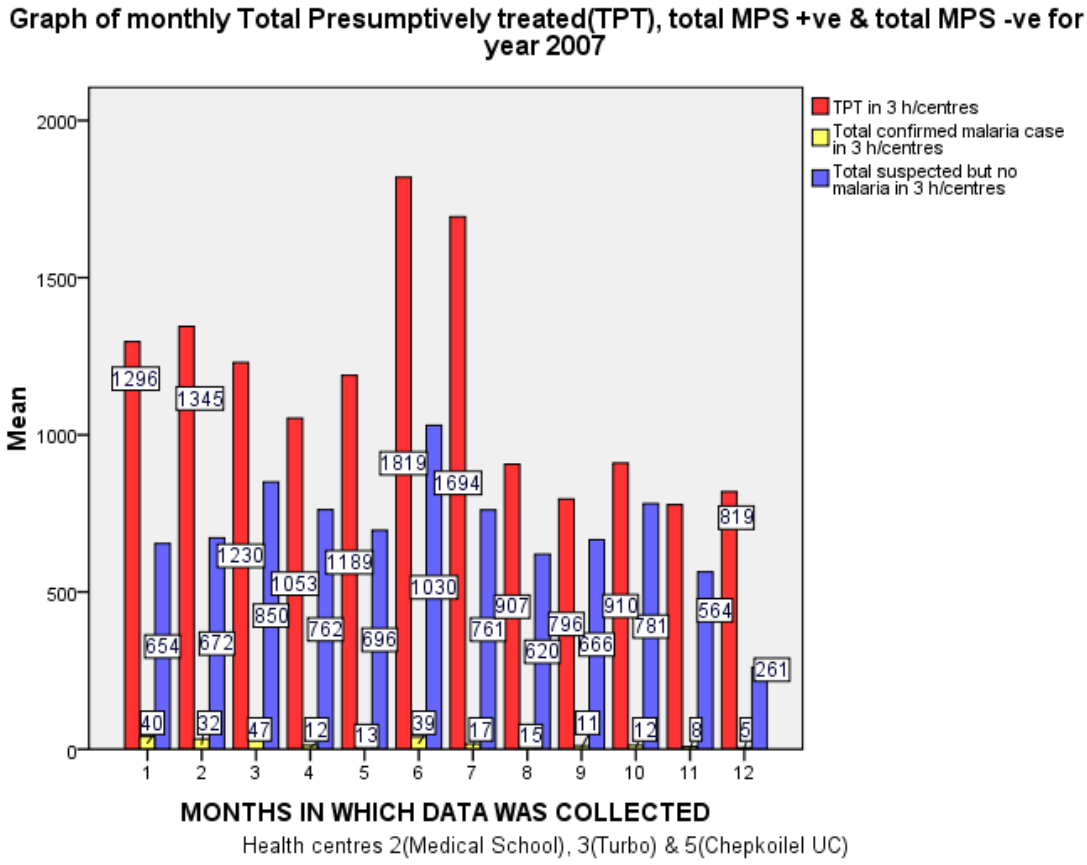
Health Centre	Duration	Presumptively treated malaria (%)	% MPS negative (Suspected malaria)	% MPS positive (Confirmed malaria)	**Total Population=N (%)**
Turbo	2001-2007	70,466 (64.05%)	31,052 (28.22%)	10,031 (7.73%)	**111,549 (100.0%)**
Kapkangani	2010	2161 (47.44%)	2161 (47.44%)	233 (5.12%)	**4,555 (100.00%)**
Kesses (MU)	2008-2009	4070 (50.86%)	3157 (39.45%)	775 (9.69%)	**8,002 (100.00%)**
Med.School (MU)	2007-2010	956 (42.09%)	11,583 (50.99%)	1,573 (6.92%)	**22,718 (100.00%)**
University of Eldoret (UoE)	2001-2007	3,185 (47.49%)	3,194 (47.63%)	327 (4.88%)	**6,706(100.00 %)**
Grand Total Population	2001-2010	**89,444 (58.26%)**	**51,147 (33.31%)**	**12,939 (8.43%)**	**153,530 (100.00%)**

Appendix 7: Analysis of Malaria Diagnosis across Rural health centres in the highlands of western Kenya



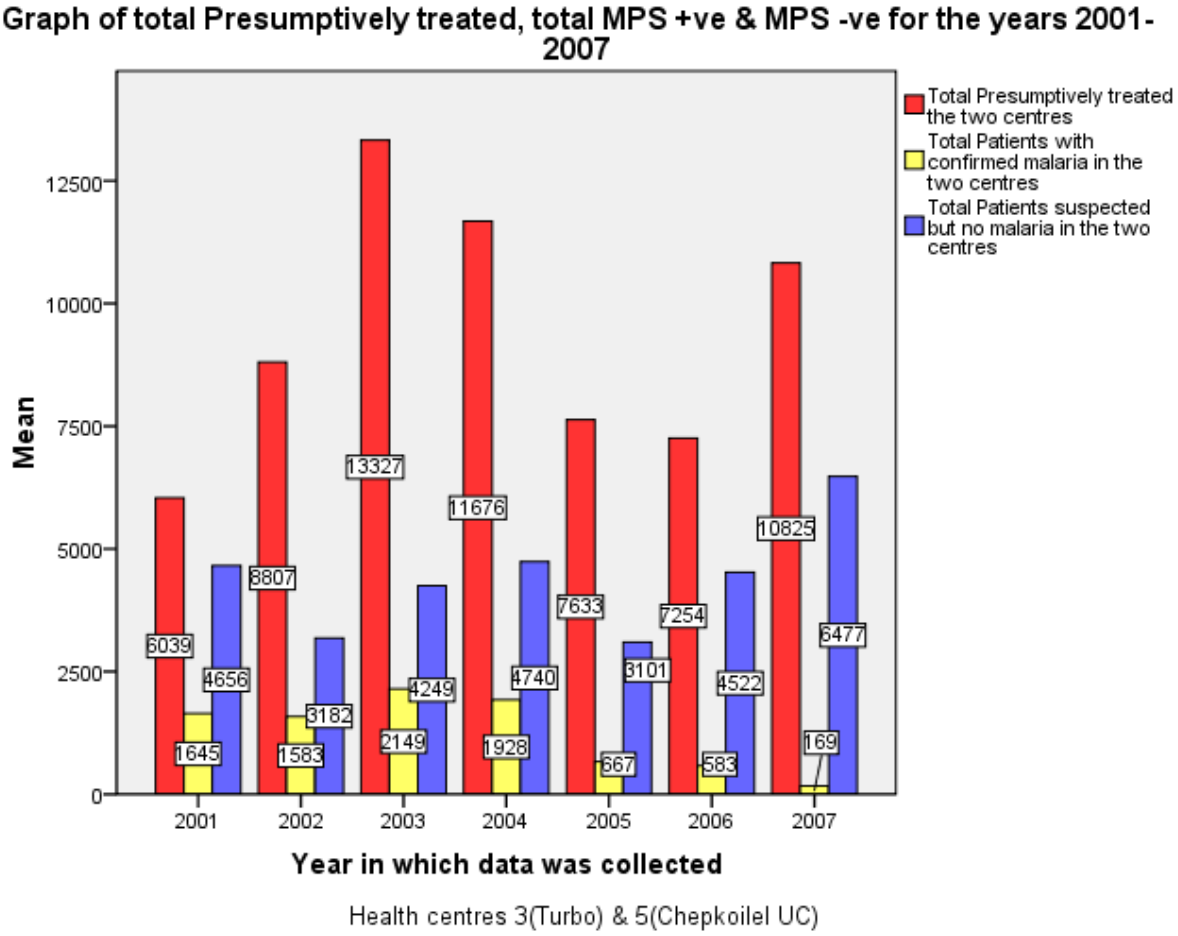
A paired sample T- test was carried out to test the equality of means between the total presumptively treated and the total confirmed cases of malaria from three health centres (Medical School, Turbo and Chepkoilel) across the year 2007. Total population (N) = 9771. Results showed that across the three health centres the proportion of presumptively treated patients was significantly higher than those microscopically confirmed to have malaria (mean=1.132E3; std.deviation=336.130; std. error mean=9.703E1; 95% confidence interval of the differences: 918.517-1.346E3; p<0.0001).

Appendix 7A: **Figure 4.2: Percentage distribution of patients evaluated for possible Malaria in Three Rural health centres in western Kenya highlands during 2007.**

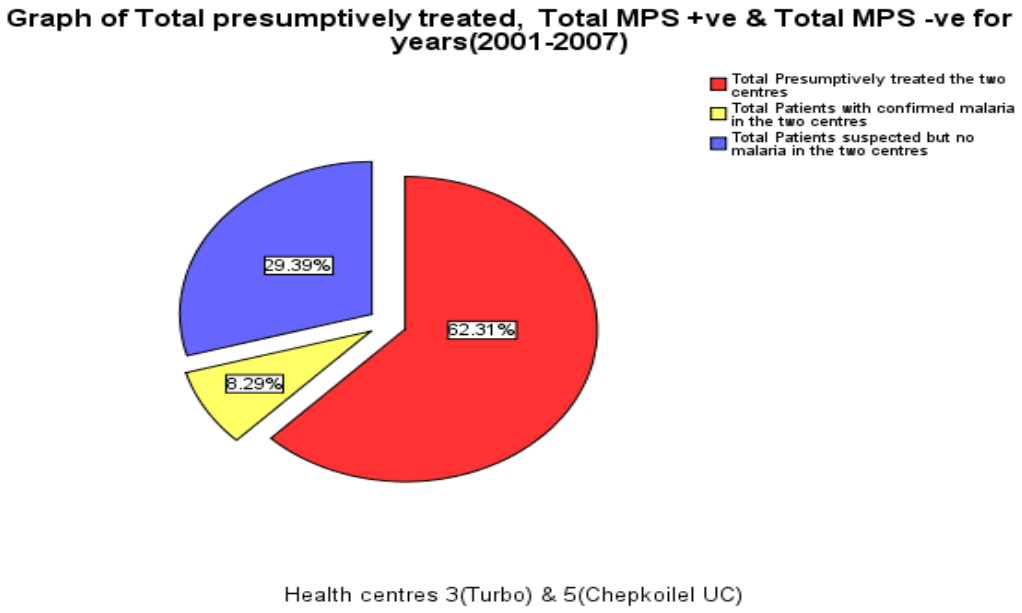


A paired sample T- test was carried out to test the equality of means between the total presumptively treated (TCT) and the total confirmed cases of malaria from three health centres (Medical School, Turbo and Chepkoilel) across the year 2007. Total population (N) = 9771. Results showed that across the three health centres the proportion of presumptively treated patients was significantly higher than those microscopically confirmed to have malaria (mean=1.132E3; std. deviation=336.130; std. error mean=9.703E1; 95% confidence interval of the differences: 918.517-1.346E3; p<0.0001).

Appendix 7B: **Figure 4.3: Monthly distribution of patients evaluated for possible malaria in the three selected health centres within western Kenya highlands during 2007.**

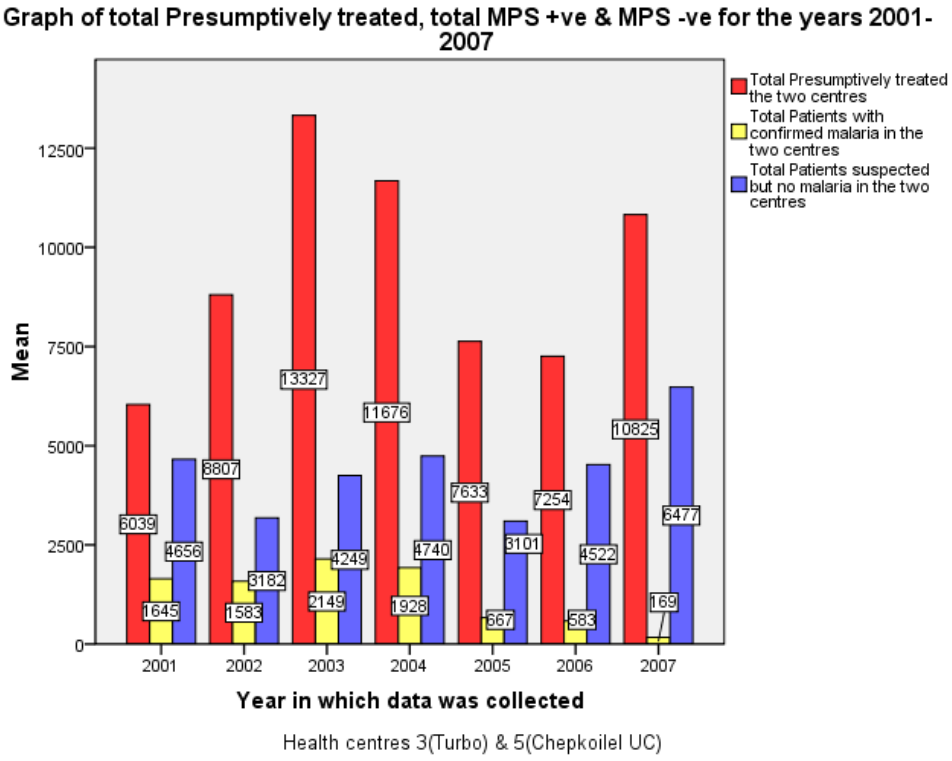


Appendix 7C: **Figure 4.4: Percentage Distribution of patients evaluated for possible malaria diagnosis in Turbo and University of Eldoret Health Centres in western Kenya between 2001 and 2007.**



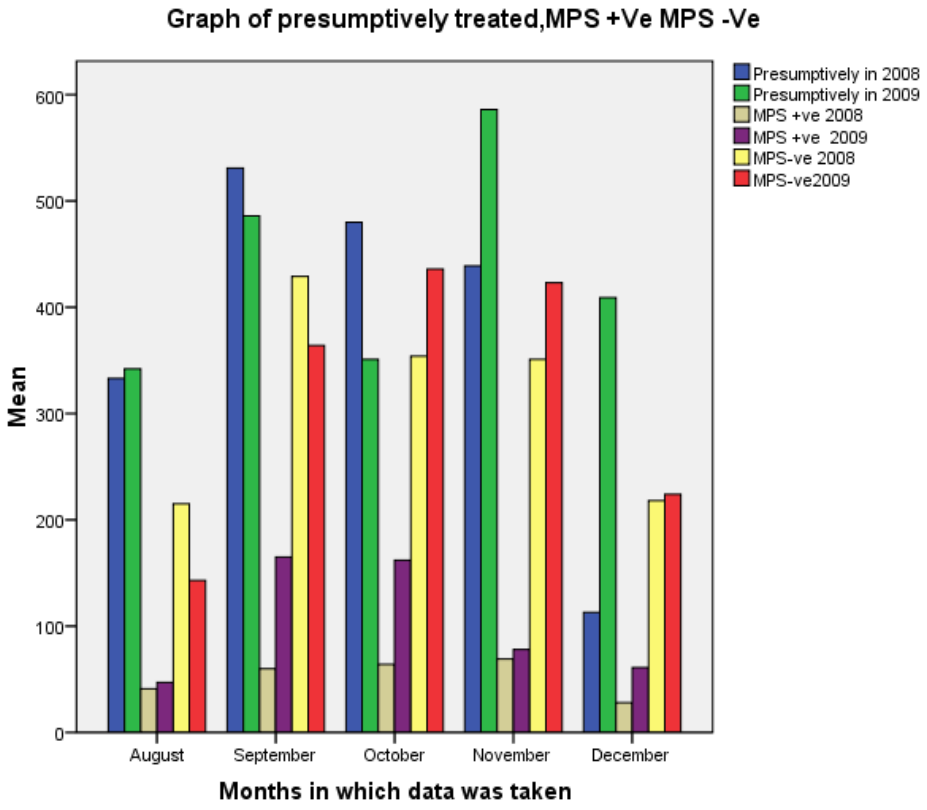
A paired sample T- test showed a highly significant statistical difference (p<0.0001) between those confirmed and those presumptively treated for malaria in the two health centres. However, there was no statistical correlation between the suspected and those confirmed to have malaria in the two health centres (p = 0.415). Total population (2001-2007) studied in the two health centres (n) = 118,255.

Appendix 7D: **Figure 4.5: Percentage Distribution of patients evaluated for possible malaria diagnosis in Turbo and University of Eldorete Health Centres in western Kenya between 2001 and 2007.**



A paired sample T- test was carried out to test the equality of means between the total presumptively treated and the total confirmed cases of malaria across the years 2001-2007 in Turbo and Chepkoilel health centres and results showed a highly significant difference between the presumptively treated and the confirmed malaria (mean=8119.571; std. error mean=932.869; 95% confidence interval of the differences: 5836.924 - 10402.219; p <0.001). Total population (2001-2007) studied in the two health centres (n) = 118,255.

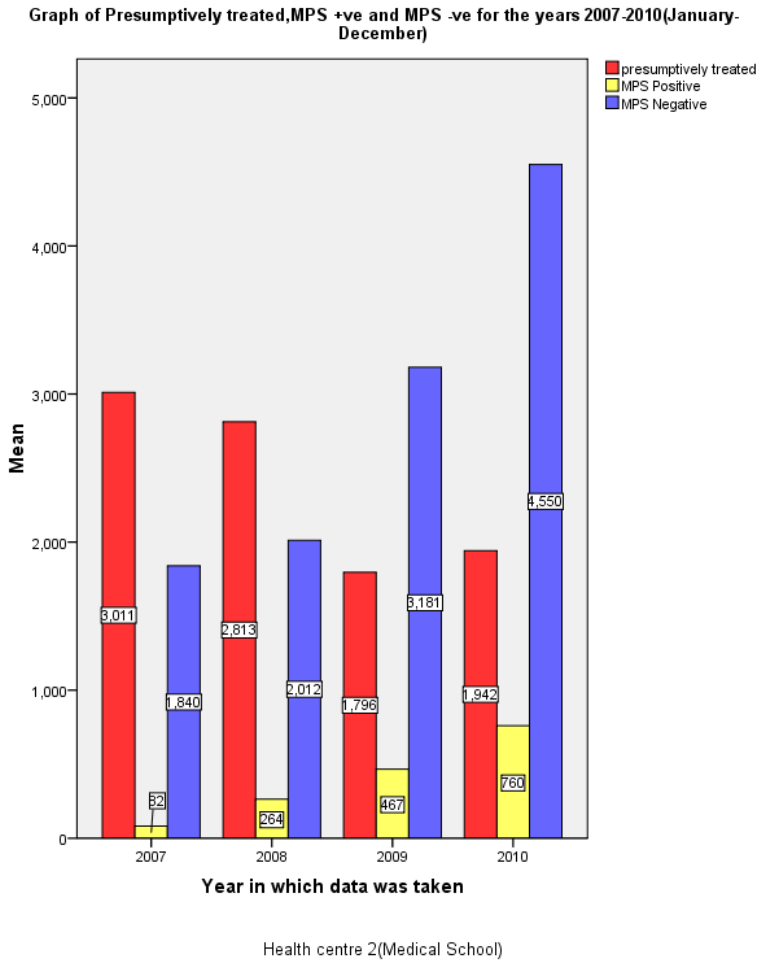
Appendix 7E: **Figure 4.6: Annual Distribution (2001-2007) of patients evaluated for possible malaria in the western Kenya highland-based Turbo and University of Eldoret Health Centres**



Key: MPS positive= Malaria Parasites positive (present); MPS negative= No malaria parasites.

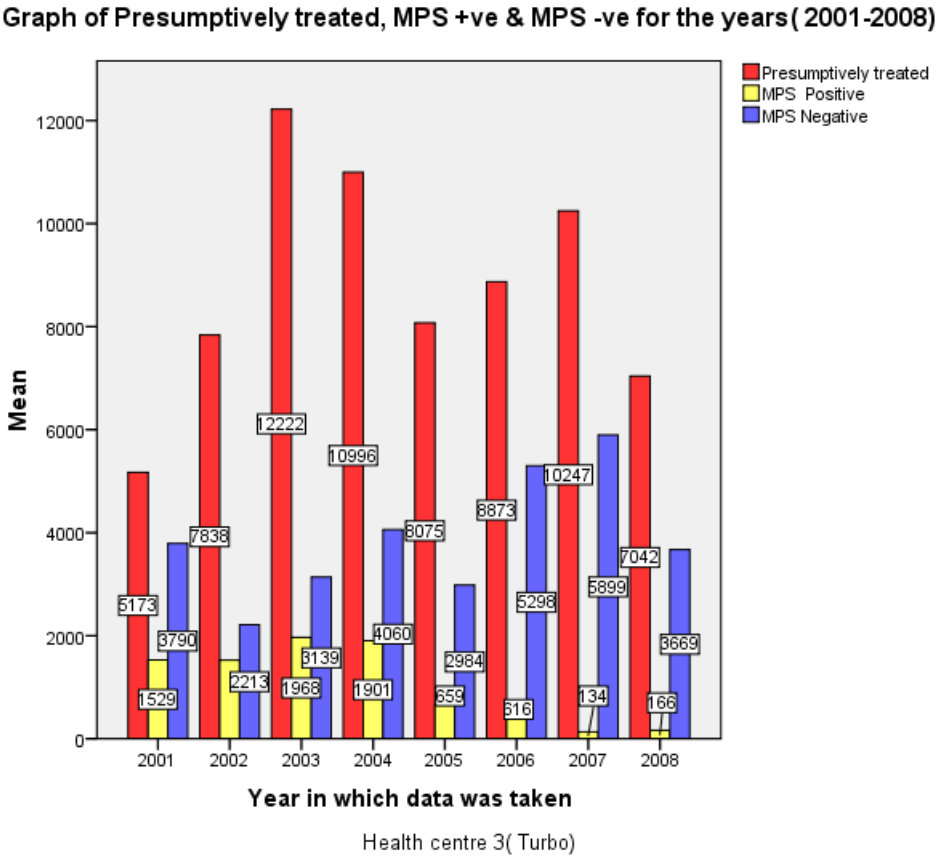
Total population (n) of Main Campus (Kesses) health centre during study period (2008-2009) was 8,002.

Appendix 7F: **Figure 4.7: Graphical Presentation of the Distribution of patients evaluated for malaria within Main Campus (Kesses), Moi University (2008 and 2009).**



Total population (n) =22,718.*Appendix 7G*: **Figure**

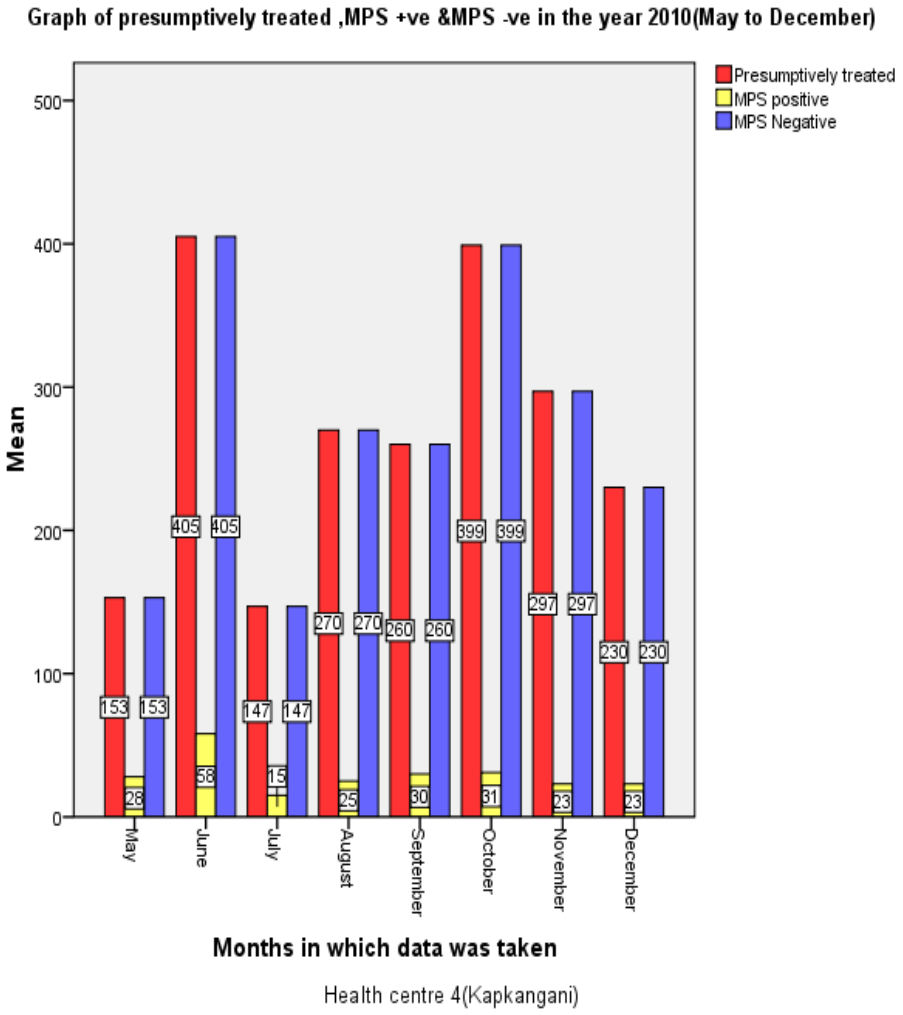
4.8: Graphical Presentation of patients evaluated for malaria within Medical School Health Centre, Moi University (2007-2010).



Key*:* MPS positive= Malaria Parasites positive (present); MPS negative= No malaria parasites.

Total population (n) = 111,549.

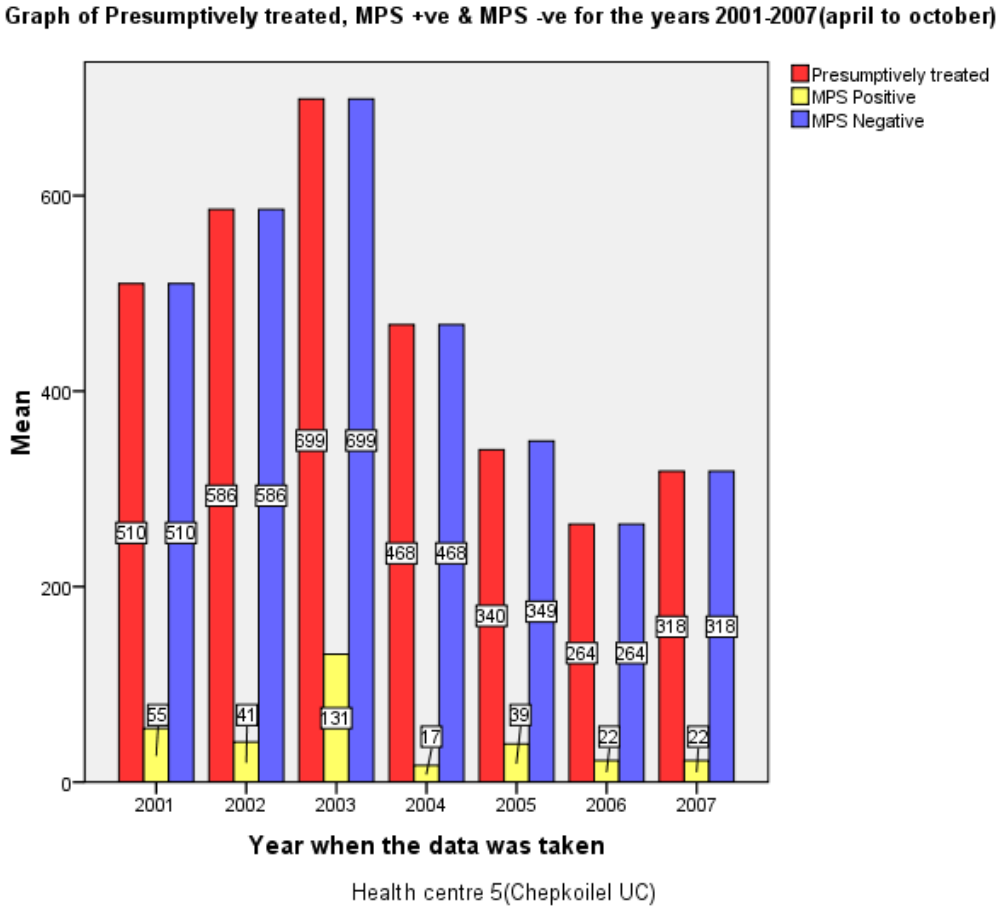
Appendix 7H: **Figure 4.9: Analysis of malaria diagnosis outcomes within Turbo Health Centre (2001-2008).**



Key: ***MPS positive= Malaria Parasites positive (present); MPS negative= No malaria parasites***.

Total population (n) =4,555.

Appendix 7I: **Figure 4.10: Graphical Distribution of patients evaluated for malaria within Kapkangani (2010).**



Total population of patients (n) evaluated at University of Eldoret Health Centre during 2001-2008 was 6706.

Appendix 7J: **Figure 4.11: Percentage Distribution of patients evaluated for possible malaria diagnosis within the University of Eldoret Centre (2001-2008).**

Appendix 8: Informed Consent Agreement Form for Parents/Guardians of Children recruited for the Research

I, Prof/Dr/Mr/Mrs/Miss……, the parent/guardian of ……. Aged……months/years *(Name and Age of Child)*, do hereby give permission/consent for my child to be included in the approved research entitled "*Correlation of Immunochromatic Rapid diagnostic test with Childhood Malaria Clinical features in Western Kenya Epidemic malaria-prone highlands.*" I have fully understood what the study is all about, the procedures to be used, the benefits, risks, hazards and discomforts, associated with the procedures to be done. I have been given an opportunity to ask questions and seek clarification on this investigational study, and the questions have been answered to my full and complete satisfaction. However, I have been made to understand that should I have any other questions concerning the rights of my child/the child under my care during the current research session, as a research subject, I may contact the Chairman, Secretary or any other official of the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital, P.O Box 3 or 4606, Eldoret, Tel. (053)33471/2/3. I accept that the investigator(s) may take blood samples from my child for the tests they need to do. I understand that I may, at any time during the course of this study, revoke my consent and withdraw from the study without any penalty or loss of treatment benefits to my child/the child under my care during the current research session.

All the issues concerning this research have been explained to me in …….language, which I clearly understand.

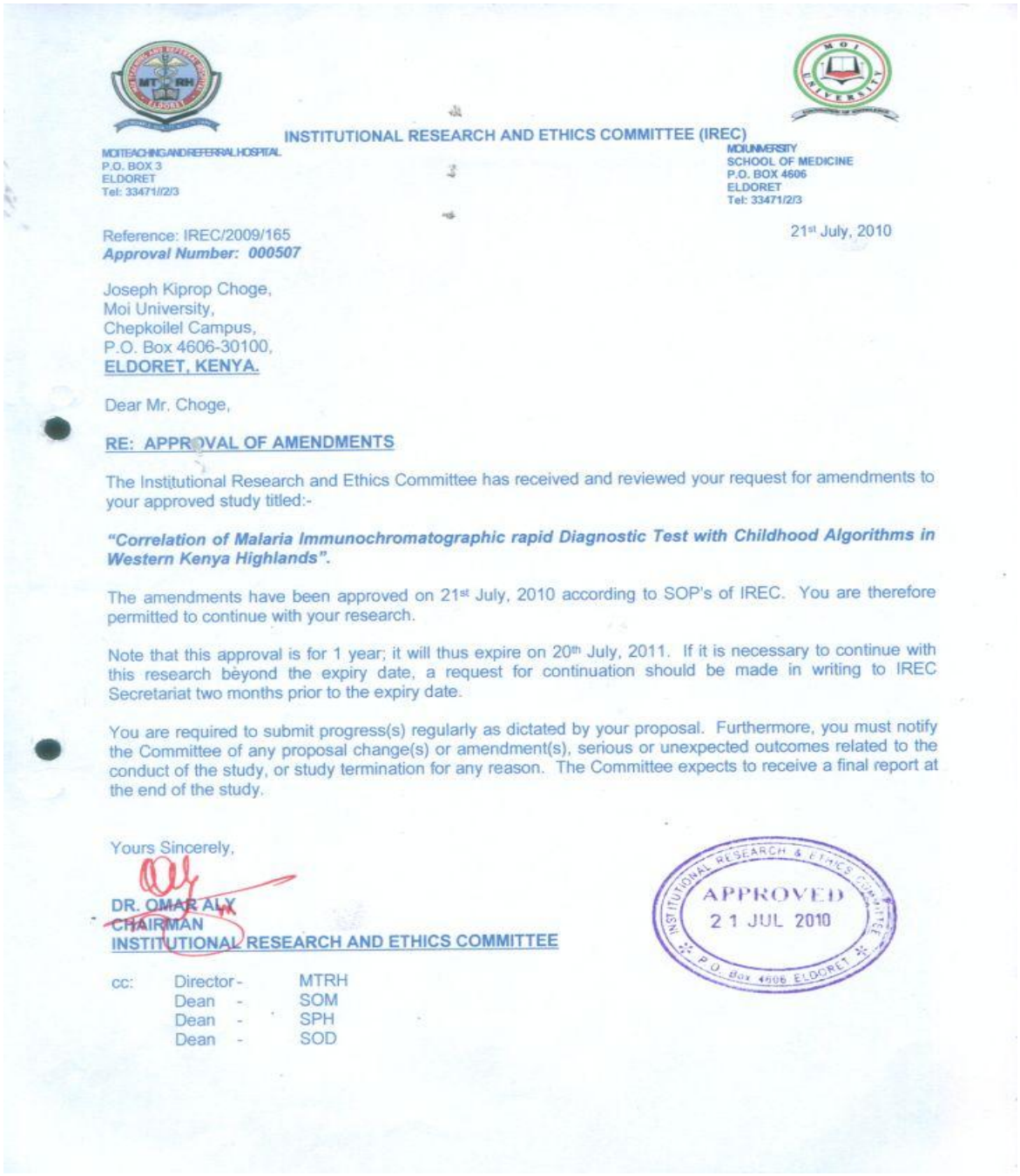
Name and Signature of

Parent/Guardian……Date……

Name of Principal Investigator: Joseph K. Choge

Signature of Principal Investigator……...Date……

Appendix 10: IREC Approval Letters for Field Rearch



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2009/165
Approval Number: 000507

21st July, 2010

Joseph Kiprop Choge,
Moi University,
Chepkoilel Campus,
P.O. Box 4606-30100,
ELDORET, KENYA.

Dear Mr. Choge,

RE: APPROVAL OF AMENDMENTS

The Institutional Research and Ethics Committee has received and reviewed your request for amendments to your approved study titled:-

"Correlation of Malaria Immunochromatographic rapid Diagnostic Test with Childhood Algorithms in Western Kenya Highlands".

The amendments have been approved on 21st July, 2010 according to SOP's of IREC. You are therefore permitted to continue with your research.

Note that this approval is for 1 year; it will thus expire on 20th July, 2011. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Yours Sincerely,

DR. OMAR ALY
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
21 JUL 2010
P.O. Box 4606 ELDORET

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2010/18
Approval Number: 000517

10th May, 2010

Choge Joseph Kiprop,
Moi University,
Health Services Department,
P.O. Box 1125-30100,
ELDORET.

Dear Mr. Choge,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:

"Period Prevalence of Selected Diseases at Moi University and Turbo Health Centres during 1998-2009."

Your proposal has been granted a Formal Approval Number: **FAN: IREC 000517** on 10th May, 2010. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 9th May, 2011. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Yours Sincerely,

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
10 MAY 2010
P.O. Box 4606 ELDORET

DR. OMAR ALY
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2010/18
Approval Number: 000517

10th May, 2010

Choge Joseph Kiprop,
Moi University,
Health Services Department,
P.O. Box 1125-30100,
ELDORET.

Dear Mr. Choge,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:

"Period Prevalence of Selected Diseases at Moi University and Turbo Health Centres during 1998-2009."

Your proposal has been granted a Formal Approval Number: **FAN: IREC 000517** on 10th May, 2010. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 9th May, 2011. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Yours Sincerely,

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
10 MAY 2010
P.O. Box 4606 ELDORET

DR. OMAR ALY
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD