COMPARISON OF MALARIA RAPID DIAGNOSTIC TESTS AT HEALTH FACILITIES AND COMMUNITY LEVEL IN SIAYA COUNTY KENYA

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I56/10109/08

A thesis submitted in partial fulfillment of the requirement for the award of the degree of Masters of Science (Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

December, 2019
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or other award

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DEDICATION

I dedicate this work to my sister Veronicah Indosio and my brother Elisha Ongoya. Your continued support, prayers and encouragement has enabled me reach this far and produce this thesis.
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To my supervisors: Dr. Fathiya Khamis, Dr. Margaret Muturi and Dr. Simon Kariuki, thanks very much for your insights, critique and for all your support. I thank you for allowing me to take this project in this direction and encouraging me to believe in myself. I have tremendously grown in the process and I am very grateful.

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<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-based Combination Therapy</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CD4+</td>
<td>Cluster of differentiation 4</td>
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<tr>
<td>CSP</td>
<td>Circumsporozoite Protein</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DOMC</td>
<td>Kenya Division of Malaria Control</td>
</tr>
<tr>
<td>EIR</td>
<td>Entomological Inoculation Rate</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HDSS</td>
<td>Health Demographic Survey System</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HRP2</td>
<td>Histidine-Rich Protein-2</td>
</tr>
<tr>
<td>IFAT</td>
<td>Immuno-Fluorescence Antibody Test</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
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<tr>
<td>IPTc</td>
<td>Intermittent Preventive Treatment in Children</td>
</tr>
<tr>
<td>IPTi</td>
<td>Intermittent Preventive Treatment in Infants</td>
</tr>
<tr>
<td>IPTp</td>
<td>Intermittent Preventive Treatment in Pregnancy</td>
</tr>
<tr>
<td>ITNs</td>
<td>Insecticide-Treated Bednets</td>
</tr>
<tr>
<td>IRS</td>
<td>Indoor Residual Spraying</td>
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<tr>
<td>KDHS</td>
<td>Kenya Demographic Health Survey</td>
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<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>KMIS</td>
<td>Kenya Malaria Indicator Survey</td>
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<tr>
<td>LAR</td>
<td>Legally Acceptable Representative</td>
</tr>
<tr>
<td>LBW</td>
<td>Low Birth Weight</td>
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<tr>
<td>LLIN</td>
<td>Long-Lasting Insecticidal Nets</td>
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<tr>
<td>MTI</td>
<td>Malaria Transmission Index</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
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<tr>
<td>NAI</td>
<td>Naturally Acquired Immunity</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PDA</td>
<td>Personal Digital Assistant</td>
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<tr>
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<td><em>Plasmodium falciparum</em> Histidine-Rich Protein 2</td>
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<td>pLDH</td>
<td><em>Plasmodium</em> Lactate Dehydrogenase</td>
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<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood Cells</td>
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<tr>
<td>RBM</td>
<td>Roll-Back Malaria</td>
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<tr>
<td>RDT</td>
<td>Rapid Diagnostic Test</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>SAS</td>
<td>Statistical Analysis Software</td>
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<tr>
<td>SP</td>
<td>Sulfadoxine-Pyrimethamine</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>Th</td>
<td>Helper T cells</td>
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<tr>
<td>µl</td>
<td>Microlitre</td>
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<tr>
<td>UNICEF</td>
<td>United Nations Children's Fund</td>
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<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Malaria remains a major public health problem affecting an estimated 40% of the world’s population and resulting in more than 650,000 deaths worldwide every year. The emergence and rapid spread of parasite resistance to affordable, easy to use and widely available anti-malarial drugs such as chloroquine and sulfadoxine-pyrimethamine (SP) has prompted many countries in endemic areas to switch to the more effective artemisinin-based combination therapies (ACTs). To mitigate against the emergence of resistance and improve patient care, the World Health Organization (WHO) recommends parasitological confirmation by microscopy or Rapid Diagnostic Tests (RDTs) before treatment. Malaria diagnosis by microscopy has many challenges including need for electricity, well trained staff and high quality reagents which are often lacking in areas where malaria is endemic. In such areas RDTs provide an alternative diagnostic method since they do not require highly trained personnel and can be performed by the community health workers. However, there is limited information in the performance characteristics (sensitivity, specificity, positive and negative predictive values) of RDTs when performed in health facilities or in the community. In this study, the performance characteristics of SD Bioline malaria Ag P.f/ pan RDT in a cross-sectional survey was compared to that from four health facilities in an area of high malaria transmission of Siaya County, western Kenya. Expert microscopy was used as a gold standard and factors affecting their accuracy were explored using statistical analysis software (SAS) version 9.2. A total of 1223 children below five years; 604 from cross sectional survey and 619 attending four health facilities (HFs), were included in the study. Sensitivity and specificity of RDTs in the cross sectional survey were 96.2% and 48.1% respectively giving positive predictive value 49.9 and negative predictive value of 99%. The health facility study had a sensitivity of 99.5% and specificity of 54.4% with positive predictive value 84% and 98% respectively. The difference in RDT sensitivity and specificity between cross sectional survey and at HFs was statistically significant (p=0.001). The health facility prevalence of Plasmodium spp. infection was 70.60% by microscopy and 83.68% by RDT while in the cross sectional survey the prevalence was 34.93 % by microscopy and 67.38 % by RDT. The result of this study showed that there was a difference in the performance characteristics of RDTs carried out in health facilities and at the cross sectional survey. However, when the analysis of sensitivity is based on parasite density, the performance of RDTs in both health facility study and during cross sectional survey is comparable to expert microscopy. Deployment of RDTs, coupled with supportive supervision can potentially reduce misdiagnosis of malaria as a result of either over diagnosis or under-diagnosis.
CHAPTER ONE

1 INTRODUCTION

1.1 Background information

In the tropics and subtropics malaria is still a major global health challenge (Boyce et al., 2018). Children below five years are the most susceptible accounting 67% of all deaths in 2017; which translates to 30 deaths of children every one hour (WHO, Malaria report, 2018). Pregnant women in endemic areas suffer mainly due to malaria associated complication mainly anaemia (UNICEF and Roll Back Malaria, 2009; WHO, Malaria report, 2018). Currently, malaria management strategy emphasises early diagnosis and treatment with artemisinin-based combination therapies (ACTs) together with intermittent preventive treatment of malaria for pregnant women (IPTp), control of mosquito vectors using insecticide-treated bed-nets (ITNs) and indoor residual spraying (IRS) and containment of epidemics (Drame et al., 2010a; WHO, Malaria report, 2018).

Wide scale deployment of these interventions have led to rapid decline in the threat of malaria in some malaria endemic areas prompting optimism that malaria can be eliminated or eradicated if these control efforts are sustained (United Nations Children's Fund-UNICEF and Roll Back Malaria, 2009; WHO, Malaria report, 2011). However, these control efforts are faced with challenges including threats of parasite resistance to anti-malarial drugs, change in vector behaviour and emergence of vector resistance to insecticides, sustainability, population movement, political unrest, environmental changes and weak health infrastructures in areas where malaria is endemic (WHO, 2009). These challenges threaten the gains in malaria control and hence continued efforts are needed in
order to overcome the challenges and reduce malaria burden to a level that it will no longer be a major a public health problem (Nkonya et al., 2016).

In highly endemic areas such as western Kenya where there is high infection rate despite the use of prevention strategies such as ITNs, early diagnosis and prompt treatment with effective ACTs play an important role in malaria control (Hamel et al., 2011). However, the implementation of malaria preventive measures in these areas are problematic due to weak health infrastructures, inadequacy of functional laboratories, shortage of trained laboratory staff and insufficiency of reagents (Boyce et al., 2018). These have led to over diagnosis of malaria and treatment of all fever cases as malaria as a result of clinical diagnosis (Omondi et al., 2017). This has contributed to poor patient management since the causes of non-malarial fevers are not treated and the over use of anti-malarial drugs could lead to emergence of parasite resistance due to drug pressure (Leslie et al., 2012).

To effectively manage malaria, there is need for accurate, easy to use and affordable tools for diagnosis of the disease (Nkonya et al., 2016). The process of developing diagnostic tools must take care of issues such as the vulnerable population of young children, pregnant women, people in endemic areas, occupational risks and seasonality of malaria vectors and infection (WHO, 2011). Most symptomatic malaria in sub Saharan Africa is treated in community health facilities, at peripheral primary health facilities and in informal health structures (Nankabirwa et al., 2009). For a malaria management strategy to be effective, it must put in place mechanisms that ensure that there is access to treatment at every health care level, and this should include private facilities and
communities to ensure that the services are closer to the targeted groups (WHO RDT Testing Report 2012).

1.2 Statement of the Research Problem

The use of fever as the basis for clinical malaria diagnosis is very common in malaria endemic areas in Africa (Nkonya et al., 2016). As a measure of improving patient management and to prevent emergence of parasite resistance to ACTs as a result of under- and over-diagnosis, the World Health Organization (WHO) and the Kenya Division of Malaria Control (DOMC) recommends parasitological confirmation of malaria infection by microscopy or RDT before initiation of treatment (WHO, 2011; Kenya Malaria Indicator Survey, 2010). It is thought that because of limitations of microscopy services in malaria-endemic regions in sub-Saharan Africa, then RDT use should be considerably greater than anti-malarial drug use because most fevers are likely to be of non-malarial aetiology (Bell et al., 2006). However, since the reverse is true, this indicates that there is a lot of wastage of drugs on patients who do not even have malaria due to misdiagnosis (Leslie et al., 2012). Recently, DOMC launched malaria diagnosis by use of RDTs during cross-sectional surveys and peripheral health facilities lacking capacity for microscopic diagnosis.

Rapid diagnostic tests make use of antigen detection and play a major part of malarial case management and form the foundation of wider measures of increasing access to malaria diagnosis services especially in areas where good quality microscopy cannot be performed because of different reasons (WHO RDT testing report 2012). Studies comparing the performance characteristics of different RDTs have demonstrated that the
sensitivity and specificity is comparable to expert microscopy (Diara et al., 2012). However, there are technical and operational issues that limit the use of RTDs and they include their accuracy and implementation in some areas (Chinkhumba et al., 2010). In addition, limitations of comparative field trials and the heterogeneous nature of malaria transmission, have limited the availability of good quality performance data that national malaria programmes require to make informed decisions on procurement and implementation (WHO RDT Testing Report 2012).

Although there have been numerous studies on the performance characteristics of RDTs in the hands of community health workers (CHWs), however, there are no studies that have been conducted to compare the performance of RDTs at health facilities and during cross sectional survey. It is expected that individuals reporting to health facilities will be symptomatic and sensitivity of RDTs will be high but specificity will be low (Chinkhumba et al., 2010). The opposite is true for the RDTs done at the community level. Low specificity leads to over-diagnosis of malaria which leads to increase cost of malarial treatment as a result of over prescription of antimalarial drugs (Zurovac et al., 2006). Poor sensitivity may lead to under-diagnosis leading to severe malaria therefore increased mortality. This has the potential of mistrusting RDTs results reported by CHWs and has implication in making treatment decisions and reporting results for program planning (Boyce et al., 2018).

1.3 Justification

Rapid diagnostic tests present an alternative malaria diagnosis method because they do not require electricity, highly skilled personnel and expensive reagents and they are
affordable, easy and rapid to use (Mouatcho and Goldring, 2013). In general, RDTs are suitable for use in poor-resource areas with limited facilities and can also be used by health care workers in the rural and urban communities (CHWs) (Diara et al., 2012). The use of RDTS is expected to bring these advantages to the diagnosis of malaria in the community health centres. In the recent years the use of RDTs in communities has seen a remarkable improvement (Boyce et al., 2018). There has been widespread use of RDTs in cross-sectional surveys in parts of South America and Asia, using the funds from the Global Fund to fight AIDS, tuberculosis and malaria. The use of RDTs has gained popularity in several African countries (WHO, 2011). This study therefore, compares the performance characteristics of SD Bioline malaria (antigen Plasmodium falciparum) Ag P.f/ pan diagnostic kit during cross-sectional survey and health facilities. The information obtained will be useful to malaria control programs as the government plans rolling out the use of RDT at the peripheral health facilities lacking laboratories.

1.4 Research Hypothesis

There is no difference in the performance characteristics of SD Bioline malaria Ag P.f/ pan diagnostic kit at health facilities and during cross-sectional survey.

1.5 Objectives

1.5.1 General Objective

To compare the performance characteristics of SD Bioline malaria Ag P.f/ pan diagnostic kit carried out in health facilities and during cross-sectional surveys in an area of perennial malaria transmission of Siaya County, western Kenya
1.5.2 Specific objectives

i) To determine sensitivity and specificity of SD Bioline malaria Ag P.f/ pan diagnostic kit during cross-sectional survey and health facilities

ii) To determine negative and positive predictive values of SD Bioline malaria Ag P.f/ pan diagnostic kit during cross-sectional survey and health facilities

iii) To compare the relationship between parasite density on the sensitivity of SD Bioline malaria Ag P.f/ pan diagnostic kit in health facilities and during cross-sectional survey.
CHAPTER TWO

2 LITERATURE REVIEW

2.1 The social economic effects of malaria

Malaria causes an estimated 219 million acute cases every year resulting in 650,000 deaths worldwide (WHO, 2018). Africa is highly affected by the disease recording 92% of all malaria infection cases and 80% deaths as a result (WHO, 2018). The disease has been associated with high morbidity and mortality in Kenya and it is reported to cause 3.5 million cases of infections annually. This translates to 30% outpatient visits, 19% hospital admissions and 3-5% inpatient deaths of which 34,000 are children under five years (Omondi et al., 2017). Malaria has far reaching implications on human life and its economic and social effects include sick children missing school, working days being lost, and tourism suffering. Malaria is self-perpetuating in the sense that the disease undermines the growth of the human and economic capital that is needed to eradicate it from the society (Kenya Demographic and House Survey, 2008-2009).

Children of below the age of five years and pregnant women are the worst affected by malaria because they are immature and have a weakened immunity, respectively (United Nations Children's Fund-UNICEF and Roll Back Malaria, 2009; WHO, 2018). Children who survive malaria episodes end up suffering from anaemia and cerebral complications that may affect their future development. In areas where the disease is endemic there is a prevalence of clinical and asymptomatic malaria, which tends to be high in young women and going through their first and second pregnancies (Boyce et al., 2018). Statistics have shown that more than 50 million women face the risk of malaria during their pregnancy (Omondi et al., 2017). Plasmodium falciparum malaria is a major concern during
pregnancy and can negatively affect the mother and the unborn baby. Such consequences include miscarriage, stillbirth, low birth weight (LBW) by intra-uterine growth restriction (IUGR) and maternal anaemia (Kamau, 2007; Rijken et al., 2012).

In sub-Saharan Africa, up to 20% of low birth weight (LBW) is caused by malaria during pregnancy and this figure shows 35% of low birth weight that can be prevented in the region (Falade et al., 2010). Malaria-induced LBW is reported to account for 62,000 to 363,000 infant deaths annually (Falade et al., 2010). Pregnant women are more susceptible to malaria partly because of the hormonal and immunological changes that take place in addition to the presence of infected erythrocytes in the placenta (Ezechi et al., 2012). In the first half of pregnancy malaria infection can cause a decrease in the diameter of the foetal head and P. falciparum malaria also interferes with utero-placental haemodynamics (Rijken et al., 2012).

Human immune deficiency virus (HIV), tuberculosis (TB) and malaria are three major public health problems that constrain development in the developing countries. There is a correlation between poverty and these diseases and their occurrence has been observed in similar geographical zones where they are major public health issues (Ezechi et al., 2012). Individuals with HIV infection are more susceptible to malaria infection than those who are uninfected (Foca et al., 2012).

This is as a result of losing pathogen-specific CD4+ T cell immunity (immune memory mechanisms) that affects immunity to exo-erythrocytic parasites, which may cause an increased parasitaemia (Foca et al., 2012). Protection against malaria infection via the
placenta depends on a Th1 environment in the placenta, with increased IFN-γ response to malarial antigens, and this response is impaired in HIV infected women (Crawley et al., 2010). In cases of pregnant women who are HIV positive with TB and malaria, the density of plasmodium in their bodies tend to be high. They also have high HIV viral load, and are more likely to suffer from maternal anaemia, low birth weight in children and high cases of infant mortality (Ezechi et al., 2012).

2.2 Causative agents of malaria

Malaria is caused by the parasitic protozoan Plasmodium during its asexual form (Getahun et al., 2010). Five main Plasmodium species have been identified in humans and they include: Plasmodium falciparum, P. ovale, P.vivax, P. malariae P.knowlesi (WHO 2018). Plasmodium falciparum is the most lethal of the five Plasmodium species worldwide, accounting for 98% of mortality and morbidity in Africa (Ongecha et al., 2011). In Africa, the most common malaria parasite is Plasmodium falciparum, which is also endemic in parts of Asia and South America. This parasite is responsible for severe malaria, and causes nearly all malaria deaths, and can also cause other consequences such as anaemia in pregnant women and low birth-weight in babies (Abba et al., 2012). Plasmodium malariae and P. ovale accounts for 1.8% of all malarial infections in Kenya often occurring as mixed infections. Plasmodium vivax accounts for up to 40-50% of infections (often mixed with P. falciparum) in the Northern and North Eastern parts of Kenya (Kenya National Guidelines for the Diagnosis, Treatment and Prevention of Malaria, 2010).
2.3 Malaria vectors

In sub-Saharan Africa, mosquito species *Anopheles gambiae*, *An. Arabiensis* and *An. funestus* complexes are the known transmitters of *P. falciparum* malaria (Kelly-Hope *et al.*, 2009; Mwangangi *et al.*, 2010). Malaria is holoendemic in the Kenya Medical Research Institute/Centres for Disease Control and Prevention Health Demographic Surveillance System area where it is transmitted by *An.gambiae sensu lato* and *An. funestus* (Amek *et al.*, 2012). *Anopheles. funestus* is the second major malaria vector and occupies a broad range of ecological niches and is highly anthropophilic (Poinsignon *et al.*, 2010).

In zones where the population of *An. gambiae and An. funestus* is high the rate of malaria transmission is very high, as they tend to breed in multiple habitats and their population rises at different times, and this tends to extend their period of malaria transmission (Kelly-Hope *et al.*, 2009). In most areas *An. funestus* has been known to keep the malaria transmission at the time when *An. gambiae* declines (Poinsignon *et al.*, 2010). Generally, during the rainy seasons *An. gambiae* increases in density while *An. funestus* dominates the habitats when the rains end and when the dry season commences (Kelly-Hope *et al.*, 2009).

2.4 Life cycle of the *Plasmodium* parasite

The life cycle of *plasmodium* involves both vector and the human phases (Figure 2.1)
2.4.1 Vector phase

The life cycle begins when some blood-stage parasites develop into male and female gametocytes (Pierce and Miller, 2009). During their blood meal mosquito vectors take up the *Plasmodium* and mate forming the diploid stage briefly. Further development happens through haploid stages before the migration from the gut region to the salivary glands (Thera and Plowe, 2012). In nearly 24 hours period, a group of parasites (ookinetes) get penetrate the epithelial cells of the midgut and move into the cells to the hemolymph, where they further divide and form an oocyst that produces thousands of sporozoites (Crompton *et al.*, 2010). In the oocyst, halving and multiplication of cells takes place by meiosis and the parasite becomes haploid (Mugyenyi, 2010). The sporozoites later own inhabit the salivary glands where they complete their development when the oocyst matures and ruptures. Each mating pair of gametocytes yields up to 1,000 infectious sporozoites, which are injected into the host to complete the transmission cycle (Thera and Plowe, 2012).
2.4.2 **Human phase**

About 10-100 sporozoites enter the blood with mosquito saliva after mosquito bite as they feed on human blood (Ngasala, 2010). The sporozoites take a few minutes to an hour to reach the liver and invade liver cells (Crompton *et al.*, 2010). The sporozoites are transformed to schizont within the hepatocytes, growing and dividing by schizogony to produce thousands of infective merozoites which occur after one week of infection.
Clinical symptoms of malaria are not apparent during liver-stage maturation. Infected hepatocytes release the merozoites during the blood-stage of the life cycle. This happens after the incubation and replication period which happens in the liver (Pierce and Miller, 2009).

Merozoites quickly invade red blood cells (RBCs) and multiply asexually, divide and grow to form trophozoites, that burst from RBCs, and reinvoke the cells in a few cycles and one cycle can take about two or three days (three days for *P. malariae*), until when (Doolan *et al.*, 2009). After a period of growth, the mature trophozoites divide (erythrocytic schizogony) producing new generations of merozoites and other parasites by products (pyrogenic molecules) into the bloodstream (Mugyenyi, 2010). The pyrogenic molecules are responsible for sequence of events leading to fever and other clinical symptoms and signs of malaria. Immediately after the release, the merozoites invade uninfected RBCs and repeat the cycle of erythrocytic schizogony (Thera and Plowe, 2012).

2.5 Immunity to malaria

Immunity to malaria is dependent on species, strain, stage and age and takes a long time to develop (Crawley *et al.*, 2010). Individuals who are exposed *P. falciparum* infection repeatedly tend to experience a case of naturally acquired immunity (NAI), which is critical to fight against the most dangerous cases of malaria, is slow in cases of mild malaria forms and is resistant to sterilization (Guinavart *et al.*, 2012). This evolutionary behaviour where individuals progress from malaria immunity to being partially immune from the disease is common in most infectious diseases (Gosling *et al.*, 2010). One key
factor that determines the rate at which immunity develops against malaria is the level of endemicity of the disease. (Omer et al., 2011). From early childhood all the way to adolescence there is a pattern of immunity development in areas where the disease is stable; this differs in areas where the disease is unstable, and where the community’s overall immunity tends to be low and there is no relation of the disease to age (Crawley et al., 2010).

Therefore, the tools that are designed to control malaria should confer protection to early infancy and during the most vulnerable first years and should not interfere with the process of developing naturally acquired immunity (NAI) (Guinavart, et al., 2012). Migration from an endemic area results in loss of immunity and development of clinical paroxysms upon return (Ellis et al., 2010). In addition, many epidemiological studies have shown that the adverse effects of malaria during pregnancy decline with increase in gravidity (Mutabingwa et al., 2005). This is believed to be partly due to a gradual build-up of natural immunity against malaria with increase in gravidity (Omer et al., 2011). With successive pregnancies, women tend to gain more immunity, therefore tend to be at high risk during their first or second pregnancies (Mutabingwa et al., 2005). However, it is difficult to achieve total sterile immunity even in the case where adults may have never migrated from the endemic zone (Guinovart et al., 2012).

2.6 Clinical manifestation of malaria

During infection the species and strain of plasmodium parasite determine the severity and course of a clinical attack, as well as; age, genetics, malaria-specific immunity, an
individual’s nutrition status and previous exposure to anti-malarial drugs (Mouatcho and Goldring, 2013). Most cases of malaria can either be uncomplicated or complicated.

2.6.1 Uncomplicated malaria

Uncomplicated malaria is quite easy to notice based on the earliest symptoms which are nonspecific and variable (WHO, 2010). Uncomplicated cases of malaria in children arise from all species of the parasite and manifest in the victims with headache, fever, vomiting, chills, anorexia and muscular aches (Thera and Plowe, 2012). Furthermore, patients experience abdominal pain and diarrhoea which is likely to be misdiagnosed as gastroenteritis, whereas symptoms of the respiratory system (tachypnoea, difficulty breathing and cough) might point at pneumonia (Crawley et al., 2010). Other symptoms include; weakness, myalgia, dizziness, nausea and pruritus (Mouatcho and Goldring, 2013; Getahun et al., 2010).

2.6.2 Complicated malaria

Complicated malaria (Severe malaria) can be detected with symptoms such as confusion, weakness, coma and other near-death complications (Crawley et al., 2010). Most of the severe malaria complications occur in non-immune subjects with *P. falciparum* malaria and involve central nervous system (cerebral malaria), pulmonary system (respiratory failure), renal system (acute renal failure) and/or hematopoietic system (severe anaemia) (Bartoloni and Zammarchi, 2012). According to WHO (2011) report, a patient is classified to be suffering from severe malaria if they present signs of cerebral malaria and respiration distress induced by metabolic acidosis. Cerebral malaria is a life threatening neurological condition that comes from severe case of *P falciparum* parasitaemia
infection where other causes of encephalopathy are overlooked (Bartoloni and Zammarchi, 2012). The clinical symptoms of the disease highly vary, but can be put into three major categories: coma with significant physiological changes (severe anaemia, metabolic acidosis, respiratory distress, shock); coma with protracted or multiple seizures, where unconsciousness might be caused by a long postictal state or by subclinical or subtle seizure activity, characterised by conjugate eye deviation, nystagmus, salivation, and hypoventilation (Crawley et al., 2010). Severe malaria mainly leads to severe malarial anaemia mainly in children and pregnant women.

2.6.3 Severe malarial anaemia

A severe case of anaemia due to malaria is defined in terms concentration of haemoglobin <50 g/L where there is *P. falciparum* infestation (Perkins et al., 2010). The World Health Organization (WHO) and Roll Back Malaria (RBM) Partnership recommend the use of anaemia as an indicator condition that can monitor the burden of malaria at community level as interventions are spread across the country (WHO, 2010). During pregnancy anaemia is a frequent condition in spite of the pre-pregnancy immunity that an individual may be having against malaria (Omer et al., 2011). This accounts for 26% of severe anaemia in pregnancy with malaria-related maternal deaths reaching up to 0.5% - 23%. Malaria is the primary cause of anaemia in pregnant women, and tends to be prevalent because of the body immunity changes that happen in the course of a pregnancy, which increases the susceptibility of pregnant women (WHO, 2011).

Children rarely die because of asymptomatic cases of severe malarial anaemia, but mortality can increase up to 30% when the anaemia condition is complicated severe
distress in the respiratory system and metabolic acidosis (Perkins et al., 2011). It is much better to use anaemia that is a bit moderate and not severe (haemoglobin <8 g/dl) that has a higher sensitivity than the use of parasite prevalence as a tool for monitoring malaria. Anaemia is a good indicator for malaria because it tends to be high in areas with high densities of mosquito vectors and malaria transmission. In addition, it is possible to measure and quantify anaemia in the field, as it has a morbidity problem, and when it becomes severe in pregnant women and children it can easily cause death if not treated (Mathanga et al., 2010).

2.7  Malaria case management

Malaria has been managed through two main strategies namely: control and treatment.

2.7.1  Control of malaria

There are ambitious global goals of addressing the malaria problem and the associated child mortality. Such targets can only yield results if there is wide coverage of effective control (United Nations Children's Fund-UNICEF and Roll Back Malaria, 2009). Malaria control strategy has been approached on mainly two fronts namely: vector control and drug-based methods, and of late the vaccination (Crawley et al., 2010).

2.7.1.1 Vector control

The control and prevention of malaria in the endemic areas such as developing countries, programs rely on the use of insecticide treated (bed) nets (ITNs) and indoor residual (house) spraying (IRS), which target the mosquito vectors (Yukich et al., 2008). A review of previous intervention trials has suggested that ITNs can reduce malaria cases by 39% to 62% and child mortality by 14% to 29% (Okumu and Moore, 2011). The use
of insecticide-treated nets has remained low despite the existing overwhelming evidence of the benefits that have been observed in the high risk areas (Hamel et al., 2011). The proportion of households in Africa estimated to own at least one insecticide-treated net rose from 17% in 2006 to 31% in 2008, with 24% of children younger than 5 years of age using an insecticide-treated net during 2008 (Crawley et al., 2010).

Indoor residual spraying (IRS) means applying insecticide solutions in areas such as walls and roofs in houses and within animal shelters with the aim of killing local mosquito vectors (Pluess et al., 2010; Hamel et al., 2011). Although indoor residual spraying has not been embraced on a large scale in some African countries, consistency in the use of the intervention has played a significant role in reducing the distribution of the vector and malaria transmission in most parts of southern Africa (Pluess et al., 2010). The success of indoor residual spraying depends on high, sustained coverage, correct timing of spraying (which should occur before the start of the transmission season), and continued vector susceptibility to the insecticide used (Okumu and Moore, 2011).

However, malaria vectors have developed resistance to some insecticides such as pyrethroids, which are impregnated on the netting material, and this is a major blow to IRS and insecticide-treated net programmes (Crawley et al., 2010). Both ITNS AND IRS have been found to be highly effective and recent evidence suggests they are very similar in terms of their effects on mosquitoes (Hamel et al., 2011; Yukich et al., 2008). In recent years, endemic countries using the two methods singly or in combination have reported significant declines in malaria related morbidity and mortality (Okumu and Moore, 2011).
2.7.1.2 Drug-based control methods

Prophylaxis and Intermittent Preventive Treatment (IPT) are two drug-based methods that are used in malaria prevention (Meremikwu et al., 2012). IPT is a method where an effective antimalarial drug is identified and its full course is administered in a specific vulnerable group at a specific time interval while not minding whether the recipients are parasitaemic or not (Crawley et al., 2010). Such a strategy aims at reducing the malaria infections and its effects in the target population (WHO, 2018). This Intermittent Preventive Treatment can be administered to pregnant women or it can also focus on infants (WHO, 2018). Prophylaxis administration of a drug is implemented such that the concentration of the drug in the blood is maintained in sufficient levels in order to prevent the growth of the parasites at specific stages of its life cycle (Meremikwu et al., 2012).

Malaria drugs need to confer protection against recurrent infections for them to be completely preventive and also fight against occurrence of new infections ideally until a new dose is administered (Meremikwu et al., 2012). Sulfadoxine-pyrimethamine has been widely used for IPT (Gosling et al., 2010; WHO, 2018). Findings of pooled data from six randomised trials have shown that the efficacy of sulfadoxine-pyrimethamine administered to infants (IPTi) that compared with placebo, the intervention had a 30.3% protective efficacy against clinical malaria, 21.3% against anaemia (haemoglobin <80 g/L or packed-cell volume <25%), and 38.1% against hospital admissions associated with malaria parasitaemia, however, it did not affect mortality (Crawley et al., 2010).
Additionally, malaria parasites are developing resistance against sulfadoxine pyrimethamine at a rapid rate thus undermining its future effectiveness and new drugs are required (WHO, 2018).

2.7.1.3 Malaria vaccine

The global war against malaria can be taken a notch higher if an effective malaria vaccine is developed (Crawley et al., 2010). The most promising vaccine is RTS,S based on one of the most dominant proteins on the sporozoite surface known as circumsporozoite protein (CSP) (Doolan et al., 2012). Although RTS,S/AS02 has a low efficacy of 53% over 10 months, which is below that of other disease vaccines, it remains one of the key milestones in the war against malaria (Crompton et al., 2010). Evidence from an ongoing multi-site phase 3 trials in seven African countries of different malaria transmission intensities shows RTS,S/AS01E vaccine has an efficacy of about 56% against clinical malaria in children of 5 to 17 months (Bedjon, P. et al., 2008).

However, many obstacles that have constrained efforts towards developing an effective vaccine for malaria include: the fact that *P. falciparum* genome is very big; having about 23 million bases of DNA organized into 14 chromosomes and about 5,000 genes (Thera and Plowe, 2012). In addition, many *P. falciparum* proteins are highly polymorphic and have redundant functions, which constrains the process of designing an effective vaccine (Crompton et al., 2010).

2.7.2 Treatment of malaria
A significant 50% of deaths from severe childhood diseases and conditions, including malaria, tend to occur within 24 hours after the patients are admitted in hospital (Mutabingwa et al., 2005). Malaria can be cured if it is identified early after accurate diagnosis followed by the right treatment of children at high risk. These steps have a positive impact in reducing illness and death, and also form the core of the current policy for controlling malaria (Abba et al., 2012). Treating uncomplicated *P. Falciparum* focuses on objectives such as curing the infection since this deters the disease from further progression to the severe state, and it also addresses the issue of morbidity in case of failure of the treatment. The treatment also focuses on preventing the development of anti-malarial drug resistance and reducing future transmissions (Crawley et al., 2010).

In reference to the above, ACTs have provided a more effective treatment as compared to chloroquine, sulfadoxine-pyrimethamine, and quinine; they are still the most widely used anti-malarial drugs (WHO, 2018). This is because artemisinin and its derivatives kill the parasites at a very high rates and specifically disable the asexual and sexual stages of plasmodium hence preventing its development in the hosts blood with two critical therapeutic effects: it prevents deterioration of the host and further transmission of the parasites (Crawley et al., 2010). Artemisinin based derivatives tend to have high tolerance, and the WHO highly recommends them to be given first priority in the treatment against *P. falciparum* and other strains such as chloroquine-resistant *P. vivax* parasites. The composition of ACTs includes articulate with either mefloquine or amodiaquine, or a combination of artemether with lumefantrine, dihydroartemisinin, and piperaquine (WHO, 2018).
2.8 Malaria diagnosis

Enhancing diagnosis with fast tests and treatment with appropriate antibiotics is one of the key strategies of the global malaria control programme (Wongsrichanalai et al., 2007). Moreover, it can also address adverse antibiotic effects because of prophylactic use of anti-malarial drugs and improve the management of pathologies other than malaria (Gerstl et al., 2010). The anti-malarial drug policy has shifted to ACTs because of the rising cases of resistance of \( P. falciparum \) to common anti-malarial drugs in most countries (WHO, 2018). If the cases of malaria accurately detected, then it reduces the misuse of ACT treatment and therefore, prevents cases of the parasites developing resistance against the drugs (Gerstl et al., 2010). Clinical diagnosis and parasitological diagnosis are the two key approaches that are commonly used (Tangpukdee et al., 2009).

2.8.1 Clinical diagnosis

This method is based on clinical symptoms of the parasites. It is less expensive, easy to carry out and with less delay in obtaining the results making it the most common method of malaria diagnosis used in rural areas in sub-Saharan Africa because it is based on clinical symptoms of the patient (Tangpukdee et al., 2009). This diagnosis tends to be unreliable because malaria symptoms are not specific and tend to overlap with symptoms of other diseases, such as typhoid and flue infections (Wongsrichanalai et al., 2007; Endeshaw et al., 2008).
2.8.2 Parasitological diagnosis

With the emergence of over-diagnosis and hence the risk of drug resistance development, WHO recommends that people should be treated of malaria after undergoing accurate confirmation of the parasites in the laboratory (WHO, 2018). Initially, this recommendation was restricted to adults and older children, but the new guidelines, released on March 9th, 2010, increase the coverage of the policy of testing first to children below five years (WHO, 2011). This method is advantageous because it enables the health workers to identify different strains of malaria parasites (Endeshaw et al., 2008).

The identification of malaria parasites is critical because it makes’ it possible to vary treatment and be more effective. In addition, when gametocytes are present they are good indicators of an active infection in cases of untreated patients and persistent infection in partially treated patients (Bell et al., 2006). Furthermore, counting of parasites is beneficial because it helps in determining severity of the disease and in patients with imported malaria (non-immunes), the evidence of any parasites in blood samples is clinically significant as it may lead to development of malaria (Chotivanich et al., 2007). The parasitological method also pins the treatment and management of the illness to a confirmatory method hence reducing over-diagnosis of malaria (Chotivanich et al., 2007). This is necessary for preserved drug supply and efficacy by decreasing exposure of parasite populations to anti-malarial drugs. This further reduces the risks of drug resistance development (WHO, 2018).

Over-diagnosis has also been observed, and it leads to prescription of malaria treatment to patients who do not have parasites hence drugs being wasted (Leslie et al., 2012).
Furthermore, it has led to serious non-malarial infections such as pneumonia and invasive bacterial disease being overlooked (Leslie et al., 2012). Parasitological diagnostic techniques have also been developed and include RDTs and the reference tests; microscopy and polymerase chain reaction (PCR) which is mostly used. Others are automated flow cytometry and serological test (Chotivanich et al., 2007). Each of these diagnostic techniques has its unique challenges that have remained unresolved despite their use.

2.8.2.1 Rapid Diagnostic Tests (RDTs)

Rapid diagnostic tests make use of antigen detection and play a major part of malarial case management and form the foundation of wider measures of increasing access to malaria diagnosis services especially in areas where good quality microscopy cannot be performed because of different reasons (WHO RDT testing report, 2012). The RDTs are work by detecting antigens that are specific to malaria parasites in a drop of fresh blood through a process called lateral flow immune-chromatography (Boyce et al., 2018). The World Health Organization (WHO) has listed 96 test kits that are sold in various commercial outlets as they meet ISO13485:2003 manufacturing standards (Abba et al., 2012). Currently available RDTs are packaged for use in different forms such as dipstick, cassette or card (WHO, 2018).

RDTs have blood antibodies that can bind to specific antigens such as histidine-rich protein-2 (HRP-2) and pan-specific or species-specific plasmodium lactate dehydrogenase (pLDH) or aldolase (Gerstl et al., 2010). Rapid diagnostic tests formed from HRP-2 only detect P. falciparum, those that have pLDH and aldolase are capable
of detecting \textit{P. falciparum} and other strains of malaria that affect humans, while other types of RDTs are based on a composition of HRP-2 and pLDH or HRP-2 and aldolase which is critical in terms of enhancing their ability to precisely detect all five species of malaria parasite that are common in humans (\textit{P. falciparum}, \textit{P. vivax}, \textit{P. ovale}, \textit{P. malariae} and \textit{P. knowlesi}) in mixed or mono-infections (Ishegoma \textit{et al.}, 2011). \textit{Plasmodium falciparum} histidine-rich protein 2 (PfHRP-2) is soluble in water and can be located inside the malaria parasites or red blood cells of the hosts. The protein is known to circulate freely or binds to antibodies in the plasma compartment (Hendriksen \textit{et al.}, 2012).

\textit{Plasmodium falciparum} histidine-rich protein 2 (PfHRP-2) is a protein that is made up of histidine- and alanine and has repetitive epitopes synthesized through the process of asexual and early sexual reproduction after which is transferred through the cytoplasm of red blood cells and the surface membrane to prepare it to accumulate in the extracellular plasma (Mouatcho & Goldring, 2013). The production of PfHRP-2 reaches its highest levels during the trophozoite stage and upto 90% of its content is released during the rapture of schizont. Upon release PfHRP-2 is dispersed becomes part of the total plasma volume. Plasma PfHRP-2 has been found to be a good measure of the total parasite burden 48-hour after the asexual phase of the parasite (Hendriksen \textit{et al.}, 2012). All \textit{Plasmodium} species that infect humans produce an enzyme called plasmodium lactate dehydrogenase which is intracellular and can be detected during sexual and asexual stages of the parasites (Gerstl \textit{et al.}, 2010).
Rapid diagnostic tests can be useful alternative to microscopy in the laboratories and can be utilized by community health workers to implement diagnosis guided treatment (Wanja et al., 2016). RDTs are useful for therapy in large scale malaria surveys because they can provide diagnosis at the point of contact and can facilitate immediate treatment when it is needed. Furthermore, they can easily overcome the challenges of human resources and technical capacity that may be demanded in large-scale surveys that require using experts and microscopes in order to ensure quality of staining and examining thousands of slides (Omondi et al., 2017). There are also other logistical challenges and costs of transportation and quality assurance that are associated with large-scale surveys (Gitonga et al., 2012). Rapid diagnostic kits are suitable for a rural area setting because users do not need a laboratory or any specialized tools to conduct the tests. Furthermore, they are not complicated to read since the results are either positive or negative and come within 15 minutes (Chinkhumba et al., 2010). Using ACTs, in combination with RDTs can be more affordable and potentially help to save costs, as long as health workers restrict anti-malarial treatment to only patients who test positive for the malaria parasite (Bisoffi et al., 2010).

One limitation of the test is that their shelf-life is quite short and need to be stored under the recommended dry conditions and away from high temperature (Drame et al., 2010a). In addition, RDTs are not efficient in cases of low parasite concentration and they are also not suitable for assessing the response of patients to treatment and false positives (especially RDTs that detect HRP-2) due to the fact that the malaria antigen may be present even after completion of malaria treatment (Diaria et al., 2012). False positives result for RDTs have the problem of overestimating the true prevalence of the parasites as
compared with expert microscopy and molecular parasite detection approaches (Gitonga et al., 2012). HRP-2 protein has the capability of remaining in the blood for approximately 28 days after commencing the treatment against malaria. The overestimation results from the failure by the RDT that detect HRP-2 antigen to separate active infection from the resolved infection because of the antigen that may be persistent (Gerstl et al., 2010). It is the presence of anti-HRP-2 antibodies in humans that explain why some people are tested of malaria despite being in a state of parasitaemia. It has also been noted that some patients have an inhibitor in their blood, which prevents the formation of the control line (Wongsrichanalai et al., 2007). Furthermore, it has also been observed that using the same RDT in different areas can generate varied results because of changes in environmental conditions, and this tends to undermine the reliability of the technique (Endeshaw et al., 2008)

2.8.2.2 Malaria diagnosis reference tests

Microscopy and polymerase chain reaction (PCR) are two important tests that are used for reference in malaria diagnosis. In conventional microscopy to detect the presence or absence of malaria, a thick or thin smear of a blood sample is examined (Endeshaw et al., 2008). It remains the baseline standard for diagnosis malaria in the laboratory (Chotivanich et al., 2007). Microscopy is efficient because it has a high degree of sensitivity and specificity to the parasites and allows differentiation and quantification of parasites at different stages, all of which are critical in assessing the severity of the disease and the recommendation of the best treatment option (Wongsrichanalai et al, 2007).
The use of microscopy for *P. falciparum* is constrained by factors such as low reproducibility, changing sensitivity and the high rate of false-positives, which is unacceptable (Bell *et al*., 2006). In addition, the use of solar-powered microscopes was viable, but was limited by insufficient sunlight, especially at the peak season when malaria transmission is high (Diara *et al*., 2012). Furthermore, for this method to succeed there is need for trained personnel, and unfortunately they can be scarce in the rural areas where malaria infections are high (Tangpukdee *et al*., 2009). Moreover, in some areas, the laboratory equipment may be missing or may not be in a working condition, quality control measures, staff training and supervision, may be present, but inadequate (Bell *et al*., 2006).

DNA/RNA forms the basis for Polymerase chain reaction (PCR), as one of the methods that is also utilized in malaria diagnosis (Chotivanich *et al*., 2007; Buppan *et al*., 2010). Polymerase chain reaction is very sensitive and useful in detecting malaria parasites (for both regular peripheral and placental) particularly for malaria cases that are associated with low parasitaemia or when they manifest with other infections (Tangpukdee *et al*., 2009). It is however not certain to determine if detection of low-level parasitaemia makes PCR suitable for malaria diagnosis because sub-microscopic parasitaemia do not have any known clinical significance and in some endemic areas there is a high occurrence of sub-microscopic infections that do not show symptoms (Abba *et al*., 2012). Its main disadvantages are: complex methodologies, being expensive and requires specially trained technicians who may not be found in areas where resources are scarce (Bell *et al*., 2006). The method is used in research laboratories for confirmatory tests of malaria infection, to male follow-ups of responses to therapy and proper identification of
parasites that are becoming resistant to drugs and case of relapse (Tangpukdee et al., 2009). In addition, it can be useful for identifying species in cases where counts are very low or samples have been deteriorated (Chotivanich et al., 2007). Microscopy is used as reference diagnostic method due to its use in our hospitals.
CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study area

This study was conducted in Karemo Division, Siaya County in western Kenya (Figure 3.1). The area covers 168 villages each with 30-100 homesteads. The area is typically rural characterised with poverty and an estimated two-thirds of the population living below the poverty line (Ombok et al., 2010). Over 95% of the people are of Luo ethnicity and they depend on fishing and subsistence farming; they grow mainly maize, sorghum and groundnuts for subsistence (Hamel et al., 2011). The area experiences rainfall throughout the year, with the heavy rains falling in March to May and the short rain period in October to November (Ombok et al., 2010). Malaria transmission in Western Kenya is intense and perennial, with seasonal peaks in April to July and November to December (Hamel et al., 2011).
Malaria infection in the area is mainly due to *P. falciparum* with entomological inoculation rate of approximately 60 to 300 infective bites per person per year (Obonyo *et al.*, 2007). However, there is high ITNs coverage since the late 1990s, when a study on the efficacy of ITNs was conducted in this area and subsequent free ITNs distribution through mass ITN campaigns at antenatal care clinics and through government subsidized schemes in selected commercial outlets (Hamel *et al.*, 2011). According to the KDHS (2008), Nyanza has a HIV/ AIDS seroprevalence of (15%) which is twice as high as the national average of 7% at that time. The four health facilities from the area that were used
for the clinical data collection are; Siaya District hospital, Ting’wangi health centre, Ng’iya health centre and K’ogelo health centre (Figure 3.1).

3.2 Study design

This study is divided into two; health facility study and cross-sectional survey; each independent of the other although analysing similar tests.

3.2.1 Study population

The number of children below five years in the two studies was as follows:

3.2.1.1 Cross-sectional survey

All members of the selected home steads above two months and below five years of age and who were not participating in a malaria vaccine trial were included in the survey. A total of 604 children were screened in the whole exercise.

3.2.1.2 Health facilities

Participants were children below five years enrolled in a malaria vaccine trial by KEMRI/CDC. In this study, 619 children were screened in the four health institutions (Siaya Distric Hospital, Ting’wangi and Ng’iya Health centres and Nyang,oma k’ogelo Dispensary) (Figure 3.1).

3.2.2 Inclusion criteria

All participants were males or females at least one month or at most five years at the time of both studies. In addition, the participants, their parent(s) and/or Legally Acceptable Representative(s) (LAR) were to comply with the requirements of the protocol as is in
consent forms. Lastly, written informed consents were obtained from the parent(s) and/or LAR of the subjects (Appendix 4)

3.2.3 Exclusion criteria

A potential participant was excluded from participation if consent/assent was not provided or not meeting inclusion criteria in 3.2.2 above.

3.2.4 Population size / Sample size determination

This research work is using data captured in two studies carried out concurrently; malaria vaccine trial and malaria parasitaemia and anaemia. The Glaxo SmithKline (GSK) Biologica RTS,S/AS01E candidate malaria vaccine is being used in Karemo for malaria trial. The annual malaria parasitaemia and anaemia surveys serve a variety of purposes, key most being to provide an accurate measurement of parasitaemia and anaemia prevalence in the Health Demographic Surveillance System (HDSS). The calculations were based on the need for complementary information on malaria transmission intensity for the RTS,S malaria vaccine trial. The primary outcomes were therefore annual estimates of malaria parasitaemia and anaemia in children aged six months to five years for the three years 2011-2013. It was aimed at enrolling 400 children ≥6 months and less than 5 years of age from different homesteads, and their household members. Accounting for 30% non-response rate, it was aimed at enrolling children and a subset of their household members from approximately 520 homesteads (Table 3.1). A sample of 400 children was therefore sufficient to detect an absolute decline in prevalence of 3.5% per year (0.40, 0.365, 0.33 and 0.295) yearly prevalence for parasitaemia with 90% power based on a two-sided test with significance level of 0.05.
Table 3.1: Sample size calculation

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>30% non-response</th>
<th>% of age group in homestead assuming 5 compound</th>
<th>Homestead needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>6months-5years</td>
<td>400</td>
<td>520</td>
<td>0.75</td>
<td>613</td>
</tr>
</tbody>
</table>

The average malaria sick cases registered per month in the health facilities from the pool of children registered for the vaccine trial is 400. Hence 400 was the number of children aimed at being enrolled in the health facilities.

3.2.5 Population sampling procedure

For the cross-sectional survey, randomly selected previously global positioning system (GPS) mapped homestead in the HDSS were used. A representative sample for this study was obtained from the HDSS population using a systematic random sampling methodology. A list of all homesteads with children less than 5 years of age was generated and ordered from the HDSS data base. To calculate the sampling interval, the total number of homesteads was divided by the sample size. A random start number was then selected from the ordered homestead listing. The sampling interval was systematically added onto the random start number until the sample size required had been achieved and all compounds having an equal probability of inclusion. The health facility study allowed all children whose parents/guardians consented as long as they conformed to the selection criteria.
3.2.6 Ethical consideration

Before conducting the cross-sectional survey and health facility study an ethical approval was obtained from the Kenya Medical Research Institute National Ethics Review Committee (KEMRI NERC) and Centers for Disease Control and Prevention Institutional Review Board (CDC IRB) was obtained (Appendix 1). Participants’ parents / guardians were provided with information about the study as contained in the informed consent form. They were asked to sign the informed consent form after understanding the contents thereof. It was clearly communicated to the parents or guardians that participation in the study was voluntary, and that a participant was free to withdraw at any time.

3.2.6.1 Potential benefits

There were no costs to participate in this study and the participants were not compensated for being part of the survey. All participants were informed about their blood results immediately. Participants who tested positive for malaria or were diagnosed with anaemia were given treatment immediately at their homes, which is a significant benefit in a setting where individuals would otherwise have to incur transport costs to procure treatment for malaria. In addition to the treatment, the guardians/parents of the participants in the health facilities were refunded their transport money every time they visited the respective hospital facilities.

3.2.6.2 Potential risks

There was a slight chance of local infection from the finger or heel prick. The participants were advised to keep the area of prick clean and report any infection to the study staff.
3.2.6.3 Privacy and confidentiality

Confidentiality of all information about the participants was maintained as required by law. The data was stored in computers at KEMRI/CDC in Kisian. Only the authorized persons had access to the data. The names and house numbers were stored with the data and will not appear on any reports.

3.3 Training of health workers

Experienced applicants were selected for the exercise of cross-sectional survey and were classified as community interviewers and sample collectors. All community interviewers and sample collectors underwent training for four days based on the KEMRI/CDC guidelines. During this training, all were inducted on data and sample collection. The sample collectors were also taught on the usage of RDTs and how the exercise is performed. Community interviewers were taught on the use of personal digital assistant (PDA) and the capturing of the required data.

3.4 Laboratory sample collection

In both cross-sectional survey and health facility study, the finger of the participant was cleaned using the ethanol swab and pricked for about 50 μl of blood to be collected. This was done after the parents/guardians consented to the process via their signatures. The health facility study was carried out during sick visits of the participants in the respective health centres. The blood sample was taken only when the participant’s body temperature was detected to be 37.5°C and above or had fever within the last 24 hours or both. The RDT testing was done immediately while the remaining blood sample was stored in EDTA for microscopy analysis.
3.5 Malaria Screening

The study used Rapid diagnostic tests (RDTs) and microscopy as a reference tests for malaria screening. All the enrolled participants went through this procedure that used their whole blood samples collected via pricking the same finger. The technicians involved in microscopic reading were blinded to RDT results in order to minimize observation bias.

3.5.1 Rapid Diagnostic Test (RDT)

The SD Bioline malaria Ag P.f/ pan diagnostic kit was used (figure. 3.2). About 5 μl of the drawn blood was put in the round sample well using a disposable specimen loop. The next step involved addition of four drops diluent assay into the square assay diluents well. The assay disrupts red blood cells for the release of pLDH and makes the strip ready for band appearance and interpretation. Two diagnostic zones of reaction that have different antibodies were in the pLDH assay. Presence of *P. falciparum* was detected in the first diagnostic zone that has its specific antibody. In the second diagnostic zone there is a pan-specific antibody found slightly above the first zone. It is this antibody that detects the pLDH of all other parasite species. A positive control is found in the top zone of the strip and has a pan-specific monoclonal antibody. The test result was then read after 15 minutes and scored positive or negative (Figure 3.2).
Figure 3.2: The figures a, b and c represents the recommended interpretation of SD Bioline malaria Ag P.f/ pan positive, negative and invalid results respectively after testing (SD.Standard Diagnostic.inc).
3.5.2 Microscopy

Microscopy was used as the reference test in laboratory analysis of malaria. The process was divided into two namely; preparation and reading of the slides and interpretation of the slides.

3.5.2.1 Preparation and reading of the slides

The microscopy (thick and thin smears) preparation and reading was done to determine the malaria parasite present. The same microscope slide was used to prepare thin and thick smears and was dried using air. Fixation of slides with thin blood smears was achieved using methanol while the thick smears were not fixed. Staining was done for each slide for ten minutes using 10% Giemsa solution. An examination of all the blood smears was done using the microscope under oil (× 1,000 magnification). To diagnose *Plasmodium* species and counting of parasite density the thick smears were used.

3.5.2.2 Interpretation of slide reading

With no observation of parasite in 100 oil-immersion fields the smears were declared as negative. Slides were scored as positive with the first reading and the second reading being all positive and when the larger of the two divided by the smaller; a value not exceeding two was the resultant. The result in this case was termed concordant and the final result was found by calculating the geometric mean of the two readings.

In some cases, slides were declared as discordant and a third independent reading was performed. This occurred first, when the result from one reading was negative and the
one from the other was positive. In addition, for high and medium positives parasitaemia results (blood parasitaemia greater than 400μl), when the highest count divided by the lowest count was greater than two. Lastly, for low parasitaemia (parasitaemia less than 400μl), the highest reading density was more than one \( \log_{10} \) more than the lowest reading. In the above cases the final result was determined either by; for cases of positive/negative discrepancy, it was wise to go by the majority. If the decision was positive, the final result was the geometric mean of the two positives or for cases of three reading, the final result was the geometric mean of the two geometrically closest readings.

### 3.6 Data entry and analysis

The data from the survey was captured by the personalised digital assistant (PDAs) that were locked with passwords such that the data could only be accessed by the data management personnel. The PDAs were collected every day and data downloaded. The data from the four health centres was recorded directly in specifically formulated forms. Data from both the health facilities field and survey was scanned (Appendices 2 and 3) and downloaded directly into an MS access data base. In order to check and clear inconsistencies data cleaning was done. Analysis of data was done using statistical analysis software (SAS) version 9.2. Sensitivity was defined as the proportion of true malaria cases (positive blood smears) that was correctly identified by positive RDTs. Specificity was the proportion of true negative malaria cases (negative blood smears) that was correctly be identified by negative RDTs.

Accuracy was the sum of true malaria cases (positive blood smears) and true negative malaria cases (negative blood smears) as a proportion of the total cases analysed. Positive
predictive value was the proportion of true malaria cases (positive blood smears) among the total number of positive RDTs. Negative predictive value was the proportion of true negative malaria cases (negative blood smears) among the total number of negative tests.

Sensitivity, specificity, positive and negative predictive values of each test was calculated for RDTs performed within the period of the two studies and estimated using microscopy as the reference standard. Sensitivity values was also stratified by category of parasitaemia (<400, 401-4000, 4001-40,000 ≥40,000) parasites/ µl) while sensitivity, specificity, positive and negative predictive values was stratified by age. Significance testing of performance characteristics of RDT for data collected in the health facilities and field survey was done by Chi-square using EPI info version 3.5. Each parameter had a 95% confidence interval (95% CI).
CHAPTER FOUR

4 RESULTS

4.1 Baseline Characteristics of the study population

All the participants in the two studies were children below the age of five years with a mean age of 32.58 and 32.68 months in cross-sectional survey and health facility study, respectively (Table 4.1). A total of 604 cases were screened in the cross-sectional survey with 407 cases being positive by RDTs while 211 by microscopy while a total of 619 cases were screened in the health facility study with 518 cases detected to being positive by RDTs while 437 by microscopy (Table 4.2; Table 4.3).

The number of positive blood smears that were identified correctly using RDT was 435 in health facility and 203 at the cross-sectional survey. The overall prevalence of *Plasmodium* spp. infection in health facility study was 70.60% by microscopy and 83.68% on the basis of RDT result alone while the cross-sectional survey yielded a prevalence of 34.93 % by microscopy and 67.38 % on the basis of RDT result. Participants screened in the cross-sectional survey had a lower geometric mean density of parasites as compared to those in the health facility study (Table 4.1).
Table 4.1: Baseline Characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Health facility</th>
<th>Cross-sectional survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases sampled</td>
<td>619</td>
<td>604</td>
</tr>
<tr>
<td>Mean age in months</td>
<td>32.68</td>
<td>32.58</td>
</tr>
<tr>
<td>BS Negative</td>
<td>193</td>
<td>393</td>
</tr>
<tr>
<td>RDT Negative</td>
<td>101</td>
<td>197</td>
</tr>
<tr>
<td>BS Positive</td>
<td>437</td>
<td>211</td>
</tr>
<tr>
<td>RDT Positive</td>
<td>518</td>
<td>407</td>
</tr>
<tr>
<td>PfGMPD</td>
<td>217117.41</td>
<td>11388.13</td>
</tr>
</tbody>
</table>

BS - blood smears, RDTs - malaria rapid diagnostic tests, PfGMPD - *Plasmodium falciparum* geometric mean parasite density (asexual parasites/μl)

Table 4.2: Cross-sectional survey data comparing RDT results against Microscopy results

<table>
<thead>
<tr>
<th>RDT results</th>
<th>Microscopy results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>203</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>211</td>
</tr>
</tbody>
</table>

Table 4.3: Health Facility data comparing RDT results against microscopy results

<table>
<thead>
<tr>
<th>RDT results</th>
<th>Microscopy results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>435</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>437</td>
</tr>
</tbody>
</table>
4.1 Performance characteristics of RDTs in the health and cross-sectional survey

The sensitivity, specificity, positive predictive value and negative predictive value of SD Bioline malaria Ag P.f/ pan diagnostic kit RDT against blood slide microscopy for all species of malaria combined are shown in Table 4.4. The specificity and sensitivity values of RDT during the health facility study were 54.4% (95% CI=46.9-61.8%) and 99.5% (CI=98.4-99.9%), respectively, with positive and negative predictive values of 84% (95% CI=80.5-87%) and 98% (95% CI=93-99.8%), respectively. The cross-sectional survey data produced a performance as follows: the specificity was 48.1% (95% CI=43.1-53.2%) while sensitivity was 96.2% (CI=92.7-98.4%). The overall positive predictive value (PPV) was 49.9% (95% CI=44.9-54.8%) while the negative predictive value (NPV) was 95.9% (95% CI=92.2-98.2%). The accuracy of the cross-sectional survey was 66.4% (95% CI=62.6-70.1%) while the health facility study had the accuracy of 86.3 (95% CI=83.3-88.9%). False positive RDT results cases were high in the cross-sectional survey (204; 50.12%) compared to those in the health facility (83; 16.02%) (Table 4.4).

Table 4.4: Performance characteristics of RDTs in the health facilities and during cross-sectional survey

<table>
<thead>
<tr>
<th></th>
<th>Health facility study</th>
<th>Cross-sectional survey</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99.5 (98.4-99.9)</td>
<td>96.2 (92.7-98.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Specificity</td>
<td>54.4(46.9-61.8)</td>
<td>48.1 (43.1-53.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PPV</td>
<td>84 (80.5-87)</td>
<td>49.9 (44.9-54.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NPV</td>
<td>98 (93-99.8)</td>
<td>95.9 (92.2-98.2)</td>
<td>0.3451</td>
</tr>
<tr>
<td>Accuracy</td>
<td>86.3 (83.3-88.9)</td>
<td>66.4 (62.6-70.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

% - percentage, PPV - positive predictive value, NPV - negative predictive value, 95% CI - 95% confidence interval, N- sample size
4.2 Effect of parasite density on sensitivity of RDT

Parasite density was categorized into four groups to determine the variation of the sensitivity of the RDT. Sensitivity increased for each of the four categories in both studies. The highest sensitivities were recorded at parasite densities of >4,000 for the health facility study and performance dropped as parasite densities decreased. Sensitivities of 100% were reached at parasite densities of >4,000 during health facility study and >40,000 during the cross-sectional survey (Table 4.5).

Table 4.5: Relationship between parasite density and sensitivity of RDTs.

<table>
<thead>
<tr>
<th>Parasite density</th>
<th>Health facility</th>
<th>N</th>
<th>Cross-sectional survey</th>
<th>N</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (95% CI)</td>
<td></td>
<td>% (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-400</td>
<td>93 (68.05-99.83)</td>
<td>14</td>
<td>90.24 (76.81-97.28)</td>
<td>37</td>
<td>0.720</td>
</tr>
<tr>
<td>401-4000</td>
<td>97.5 (86.84-99.94)</td>
<td>39</td>
<td>96.49 (87.98-99.57)</td>
<td>55</td>
<td>0.511</td>
</tr>
<tr>
<td>4001-40,000</td>
<td>100 (95.89-100.00)</td>
<td>88</td>
<td>97.89 (92.60-99.74)</td>
<td>93</td>
<td>0.171</td>
</tr>
<tr>
<td>&gt;40,000</td>
<td>100 (98.75-100.00)</td>
<td>293</td>
<td>100 (81.47-100.00)</td>
<td>18</td>
<td>1.0</td>
</tr>
</tbody>
</table>

% - percentage, 95% CI - 95% confidence interval, N-sample size
CHAPTER FIVE

5 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Overall, the sensitivity of RDTs was significantly higher in the health facility study compared to cross-sectional survey study. The cross-sectional survey had a sensitivity of 96.2% (CI=92.7-98.4%). This is similar results of 97% obtained by Chinkhumba et al. (2010) using SD Bioline Malaria Antigen P/f under field condition. The low sensitivity observed in the cross-sectional survey than health facilities study may have been caused by the following two reasons; some cases having low level parasitaemia, which was below limits that RDTs could detect thus leading to cases of false negative results and secondly, the RDTs may have had some defects or were mishandled thus interfering with their sensitivity (Endeshaw et al., 2008). In addition, the use of herbal medicine and other conventional antibiotics including anti-malarial for self-medication before testing during cross-sectional survey may have also affected the sensitivity of kits (Diara et al., 2012).

In a study carried out in Uganda using a pLDH-based RDT, a lower sensitivity of 88% was recorded (Hopkins et al., 2008). Similar results were recorded by Diara et al, (2012), with sensitivity of 89.6% (95%CI =88.1-90.9). Furthermore, Endeshaw and others (2008) using ParaScreen RDT observed a low sensitivity of 47.5% in field conditions. Ashley et al, (2009) using CareStart™ Malaria pan pLDH test for the detection of all species combined had a sensitivity of 85.1% (95%CI= 81.3-88.2). Similar reasons were thought to be the cause for the lower sensitivity for these studies. Although there is different test sensitivity in the health facilities study and cross-sectional survey, this may be surprising as the difference appears to be caused almost entirely by the different mean parasite
density in the two studies. However, if the analysis is stratified for parasite density, the sensitivity is very similar in both studies. The overall sensitivity was above 95%, the minimal level recommended by the WHO.

This high sensitivity reported may have been caused by high parasitaemia which could be detected as positive by RDT than those with low parasitaemia. This is extremely important from the point of view of patient safety. In addition, where the density of parasites was very low false negative results were recorded. When the density of parasites was over 400 parasites/μl the sensitivity shot up approaching 100% over 4,000 parasites/μl. Within the range of 1-400 parasites/μl the sensitivity is 93% for health facility and 90.24% for community survey study. This leaves out treatment for patients whose results are false negatives at low density of parasites, which might be moderately harmless.

Other studies have shown that the missed treatment among patients who had false negative malaria microscopy did not lead to severe disease (Bissoffi et al., 2010). The p value in all parasite density stratification shows no significance difference with the exception of 4001-40000 parasites/μl which may have resulted from few cases being false negative. This study showed that sensitivity increases with increase in parasite density. This is consistent with a study carried out by Diara et al. (2012) that showed positivity is proportion to parasite load.

The specificity was very low with the health facility study recording 54.4% (46.9-61.8) while 48.1% (95% CI =43.1-53.2) being recorded by the cross-sectional survey. The low rates of specificity could have been because of the persistent antigenaemia cases even
after the administration of treatment. This is in agreement with some studies that have shown that persistence in antigen leads to false positive results (Batwala et al., 2010). This was similar to 39% specificity recorded by Chinkhumba et al. (2010) using SD Bioline malaria Ag P.f/ pan diagnostic kit in field condition. Similar results were obtained from a study by Batwala et al. (2010) which showed a specificity of 74.7% using Paracheck RDT. However, in a study carried out by de Oliveira et al. (2009), 95.4% was the specificity which was caused by low parasitaemia in the study sample.

The low specificity in this study is of great concern because it could be a source that may mislead clinicians, undermine the cost effectiveness of RDTs, and may also increase the chances of drug resistance. The specificities were below 55% in all age groups in both studies which may have resulted in increased number of false positive registered in both studies. The positive predictive value was correspondingly low 49.9% (95%CI = 44.9-54.8) for the cross-sectional survey. This was as a result of false positives which accounted for 50.12% of the total negatives by microscopy. Accuracy in this study was low in the cross-sectional survey than the health facility study. This is caused by the fact that it is affected by both sensitivity and specificity hence factors affecting them affect accuracy too.

The PPV and NPV are influenced number of positive patients that are seen. The PPV tends to reduce with lower prevalence, whereas the NPV increases (Endeshaw et al., 2008). The NPV was very high in both studies because of low false negative this study had. The age stratification shows a significance difference in the NPV values at the age bracket of <36 months. This study had 83 false positives which may be attributed to
persistence of the antigen and 2 false negatives. The presence of only 2 false negatives is advantageous because the kit is able to detect all cases of infection. Both health facility and community studies had a prevalence of *Plasmodium spp.* infection of 70.60% and 34.93% respectively by microscopy. On the basis of RDT, health facility study had a prevalence of 83.68% while cross-sectional survey had 67.38%. The difference was statistically significant. This could be attributed to differences in parasite density caused by the fact that the hospital facility study had participants who were parasitaemic.

The performance characteristics of RDTs which are determined by their sensitivity, specificity, PPV, NPV and accuracy by comparing them to microscopy as a gold standard, is very critical to avoid issues of denying patients anti-malarial treatment because of false negative results and misusing drugs by treating patients who have false positive RDTs (Ishegoma *et al.*, 2011). Malaria RDTs must possess both high sensitivity and specificity in the field conditions. High sensitivity is critical in ensuring the detection of true cases of malaria and a further proper management while high specificity is important to prevent instances of false positive results (Chinkhumba *et al.*, 2010).

Missing true cases of malaria is a big threat to the lives of otherwise well children, whereas the people who are misdiagnosed with malaria often have alternative diagnosis especially for bacterial diseases, some of which are potentially fatal as they remain untreated (Ansah *et al.*, 2010). Furthermore, symptomatic treatment of malaria in areas where there are no diagnostic facilities or where health workers do not adhere to RDT results, does not motivate the health-care providers to search and treat alternative causes
of fever, and thus heightening the high drug pressure which might eventually lead to parasite tolerance/resistance to ACT (Ishegoma et al., 2011).

This study has several limitations. First, in the study it was a comparison of health facility and cross-sectional survey performance of RDT where in the health facility the tests for sick visits were employed while the cross-sectional survey had a random testing. This may have been depicted in the differences in the sensitivity because individuals reporting to health facilities are symptomatic hence sensitivity of RDTs will be high but specificity will be low. Secondly, the performance of RDTs was compared to expert microscopy that tends to focus on quality standards that are difficult to achieved under some of the conditions in health facilities as recently shown by studies conducted in Kenyan health facilities and other malaria endemic areas.

In addition, when using microscopy diagnosis as a comparative method false positive results may become an issue because of some sub-microscopic infections that are below the threshold of microscopy and cannot be detected. Studies have claimed that microscopy can miss even more than half of the sub-microscopic infections that are detected by PCR (Bendezu et al., 2010). It has also been argued that the RDTs, hypothetically may have a higher sensitivity than microscopy. If so, at least part of the cases in which the SD Bioline Malaria Antigen P/f test indicated a *P. falciparum* infection as opposed to microscopy might have been false-negative microscopy results rather than false-positive RDT results. *Vice versa*, some false-negative RDT results may have been false-positive microscopy results. Lastly, there is a possibility that community health workers may not have handled the tests in an accurate way as expected because of
the pressure from the workload brought about by the high number of patients and the period of study.

5.2 Conclusions

i) This study showed that the health facility study had higher sensitivity and specificity compared to that of cross-sectional survey.

ii) The study showed that the health facility study had a higher positive predictive value and negative predictive compared to that of cross-sectional survey.

iii) The study showed that there was no significance difference on sensitivity of RDTs on detection of different parasite density in health facilities study and on cross-sectional survey.

5.3 Recommendation from the study

i) There is difference in performance characteristics of SD Bioline malaria Ag p.f/pan diagnostic kit at health facilities and during cross-sectional survey.

ii) Deployment of RDTs, coupled with supportive supervision can potentially reduce misdiagnosis of malaria as a result of either over diagnosis or under-diagnosis.

5.4 Recommendation for further studies

i) Investigations in health facilities especially on using clinical visits data and comparing it to field survey rather than the use of sick visits data only.

ii) Further research and eventually introduction of other alternative and sensitive malaria diagnostic methods should be explored.
REFERENCES


Hendriksen, I. C. E., Mwanga-Amumpaire, J., von Seidlein, L., Mtove, G., White, L., Oloosebikan, R., Lee, S. J., Tshefu, A. K., Woodrow, C., Amos, B., Karem, C., Saiwaew, S., Maitland, K., Gomes, E., Pan-Ngum, W., Gesase, S., Silamut, K.,


Presidents malaria initiative (PMI)., (2011). Fifth Annual Report to Congress.


APPENDICES

Appendix 1: The letter of approval of the study.

KENYA MEDICAL RESEARCH INSTITUTE

KEMRI/RES/7/3/1

TO: DR. MEGHNA DESAI (PRINCIPAL INVESTIGATOR)

THROUGH: DR. JOHN VULULE, THE DIRECTOR, CGHR KISUMU

Dear Madam,

RE: SSC PROTOCOL No. 2031 (REQUEST FOR RENEWAL): EVALUATION OF THE INTRODUCTION OF MALARIA CONTROL INTERVENTIONS ON MALARIA PARASITAEMIA AND ANEMIA IN THE KEMRI/CDC HDSS

July 11, 2012

Thank you for the Continuing Review Report for the period 20th July 2011 to 8th June 2012.

This is to inform you that at the 204th meeting of the KEMRI Ethics Review Committee held on 10th July 2012, the Committee reviewed the above referenced application and made note of the following:

(a) The 2120 study participants are enrolled into the study.
(b) Overall malaria prevalence of malaria parasitemia declined steadily from 2005 until 2008 (39% to 28%). After which it steadily increases until 2011 (39%). Please explain this finding on the resurgence of malaria.
(c) The planned activity for the next project period is to continue to collect data on malaria and anaemia prevalence.

The Committee was of the opinion that the progress made in the reporting period is satisfactory and that the risk/benefit status of the study remains favourable. Consequently, the study was granted approval for continuation effective the 10th day of July 2012.

Please note that authorization to conduct this study will automatically expire on July 9, 2013. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by May 20, 2013.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is

In Search of Better Health
Appendix 2: The empty sample of the Rapid diagnostic tests sensitivity evaluation scannable form.

<table>
<thead>
<tr>
<th>Date</th>
<th>/  /</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2009</td>
</tr>
<tr>
<td>Initials of data collector</td>
<td></td>
</tr>
<tr>
<td>Name of facility</td>
<td></td>
</tr>
<tr>
<td>Study ID</td>
<td></td>
</tr>
<tr>
<td>Age of participant</td>
<td>Calculated age in months:</td>
</tr>
<tr>
<td>Had fever?</td>
<td>Yes</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>RDT name</td>
<td>PARACHEK</td>
</tr>
<tr>
<td>RDT date</td>
<td>/  /</td>
</tr>
<tr>
<td>RDT result</td>
<td>Positive</td>
</tr>
<tr>
<td>BS date</td>
<td>/  /</td>
</tr>
<tr>
<td>Blood smear result (microscopy)</td>
<td>Positive</td>
</tr>
<tr>
<td>Parasite per microlitre first reading</td>
<td></td>
</tr>
<tr>
<td>Parasite per microlitre second reading</td>
<td></td>
</tr>
<tr>
<td>Parasite per microlitre third reading</td>
<td></td>
</tr>
<tr>
<td>Form reviewed by</td>
<td></td>
</tr>
<tr>
<td>Filenum</td>
<td></td>
</tr>
</tbody>
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Rapid diagnostic tests version 1
Appendix 3: The filled sample of the Rapid diagnostic tests sensitivity evaluation scannable form

![Image of filled Rapid Diagnostic Test form]

<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
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<td>Age of participant</td>
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<td>Had fever?</td>
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<td>Positive</td>
</tr>
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<td>Parasite per microlitre first reading</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Parasite per microlitre second reading</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
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<td>Parasite per microlitre third reading</td>
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<td>3249</td>
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</tbody>
</table>

Rapid diagnostic tests version 1
Appendix 4: Consent statement

## Consent statement

Your signature below means that you voluntarily agree to participate / have your child to participate in this research study:

```markdown
The above has been explained to me and I agree to take part in the study. I understand that I am free to choose for me and my child to be in this study and that saying "NO" will have no effect on me or my child. I agree for my child's blood to be tested for malaria and anemia. I understand that relevant sections of my health records and facts collected during the study may be looked at by staff from KEMRI/CDC. I give permission for these persons to have access to my records and link them to other KEMRI/CDC studies and DSS data. I also give permission to share the facts collected through this study, without my name and address, with other studies.
```

<table>
<thead>
<tr>
<th>Adult / Mature minor providing consent for self</th>
<th>Name:</th>
<th>Signature:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent providing consent for child</td>
<td>Name:</td>
<td>Signature:</td>
</tr>
<tr>
<td>Witness*</td>
<td>Name:</td>
<td>Signature:</td>
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<tr>
<td>Study staff consenting participant</td>
<td>Name:</td>
<td>Signature:</td>
</tr>
</tbody>
</table>

*If you agree, circle “YES.”

| If you agree, circle “YES.” | YES |

<table>
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<th>Name:</th>
<th>Signature:</th>
<th>Today’s date</th>
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</thead>
<tbody>
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<td>Parent providing consent for child</td>
<td>Name:</td>
<td>Signature:</td>
<td>Today’s date</td>
</tr>
<tr>
<td>Witness*</td>
<td>Name:</td>
<td>Signature:</td>
<td>Today’s date</td>
</tr>
<tr>
<td>Study staff consenting participant</td>
<td>Name:</td>
<td>Signature:</td>
<td>Today’s date</td>
</tr>
</tbody>
</table>

* A participant or parent can sign or verbally state his/her consent in the presence of a witness who will then sign.