PERFORMANCE OF RAPID IMMUNOCHROMATOGRAPHIC TEST AND
THE EFFECT OF AGE AND PARITY ON MALARIA INFECTION AMONG
EXPECTANT WOMEN IN KERICHO COUNTY

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SCIENCES OF KENYATTA UNIVERSITY

SEPTEMBER, 2016
DECLARATION

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

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DEDICATION

This work is dedicated to my parents and the whole family for their continued support throughout my study.
ACKNOWLEDGEMENT

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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-Combination Therapy</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>CD</td>
<td>Cell differentiation</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CMI</td>
<td>Cell Mediated Immunity</td>
</tr>
<tr>
<td>CSA</td>
<td>Chondroitin sulfate</td>
</tr>
<tr>
<td>DBL</td>
<td>Duffy binding-like</td>
</tr>
<tr>
<td>df</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Dinucleotide triphosphate</td>
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<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
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<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>Flow cytometry</td>
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<tr>
<td>FN</td>
<td>False Negative</td>
</tr>
<tr>
<td>FP</td>
<td>False Positive</td>
</tr>
<tr>
<td>GMP</td>
<td>Global Malaria Programme</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HRP-2</td>
<td>Histidine Rich Protein-2</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular Adhesion Molecule 1</td>
</tr>
<tr>
<td>ICT</td>
<td>Immunochromatographic test</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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<tr>
<td>IE</td>
<td>Infected Erythrocytes</td>
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<tr>
<td>IFA</td>
<td>Immunofluorescence antibody testing</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IPTp</td>
<td>Intermittent Preventive Treatment in pregnancy</td>
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<tr>
<td>IREC</td>
<td>Institutional Research and Ethics Committee</td>
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<tr>
<td>IRS</td>
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<tr>
<td>ITNs</td>
<td>Insecticidal Treated Nets</td>
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<td>LAMP</td>
<td>Loop-mediated isothermal amplification</td>
</tr>
<tr>
<td>LBW</td>
<td>Low birth weight</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactose Dehydrogenase</td>
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<tr>
<td>LLINS</td>
<td>Long Lasting Insecticidal Nets</td>
</tr>
<tr>
<td>mAbs</td>
<td>Monoclonal antibodies</td>
</tr>
<tr>
<td>MOP</td>
<td>Malaria Operational Plan</td>
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<tr>
<td>MRDDs</td>
<td>Malaria rapid diagnostic Devices</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
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<tr>
<td>P. F</td>
<td><em>Plasmodium falciparum</em></td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PECAM1</td>
<td>Platelet endothelial cell adhesion molecule 1</td>
</tr>
<tr>
<td>PfEMP 1</td>
<td><em>Plasmodium falciparum</em> erythrocyte membrane protein-1</td>
</tr>
<tr>
<td>pLDH</td>
<td><em>Plasmodium</em> lactate dehydrogenase</td>
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<tr>
<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>RBC’s</td>
<td>Red Blood Cells</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>---------------------------------</td>
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<tr>
<td>RDTs</td>
<td>Rapid diagnostic tests</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>SP</td>
<td>Sulphadoxine Pyrimethamine</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>TDR</td>
<td>Tropical Diseases Research</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TN</td>
<td>True Negative</td>
</tr>
<tr>
<td>TP</td>
<td>True Positive</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular cell adhesion molecule 1</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>Chi square</td>
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<tr>
<td>$\mu l$</td>
<td>Microlitres</td>
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Malaria is a major contributor of ill health and death particularly among infants and pregnant women in sub-Saharan Africa. The infected erythrocytes sequester in the placenta and may be present throughout the whole pregnancy period. Although microscopy is the gold standard method currently acceptable in diagnosis of malaria in hospital, it is unreliable because during pregnancy, malaria parasites disappear from peripheral circulation and remain undetectable through microscopic examination. This technique also requires expertise to perform and it is time consuming hence difficult to implement in remote areas where malaria is more common. The ability of rapid immunochromatographic tests to detect malaria parasites sequestered in the placenta has been reported but their accuracy and sensitivity in detection of malaria among pregnant women under different ecological settings remains unclear. This study compared the performance of the rapid immunochromatographic strip test relative to microscopy using polymerase chain reaction as a confirmatory test in malaria diagnosis among pregnant women in Kericho Highlands. The effect of age and parity on malaria prevalence and parasitaemia level was also examined. Blood samples from a total of 314 pregnant women attending the antenatal clinic were screened for malaria parasites using microscopy, rapid ICT and PCR. Structured questionnaires were also administered to obtain information on the effect of age and parity on malaria prevalence and parasitaemia level. Data were analyzed using Chi-square, logistic regression analysis, odds ratio tests and Cohen kappa index computation. Out of the 314 samples analyzed by microscopy, 44 samples (14.0%) were positive for *P. falciparum* while rapid ICT diagnosed 62 (19.7%) as positive for *P. falciparum*. The sensitivity and specificity of rapid ICT was 84.4% and 96.8% respectively while that of microscopy was 68.8% and 100.0% respectively. The Cohen kappa index (κ) indicated a moderate level of agreement between results of rapid ICT and microscopy and high level of agreement when rapid ICT results were compared against microscopy and PCR results (κ = 0.517; p <0.001 and κ = 0.821; p <0.001) respectively. Chi-square analysis tests showed that parity had no significant effect on malaria prevalence (P < 0.001). Odds Ratio tests showed no significant difference in parasitaemia in infected multiparous and primiparous women (p=0.748) and that age had no significant effect on parasitaemia levels among the study population (p=478). Binary logistic regression analysis revealed that maternal age and parity are not significant predictors of malaria prevalence and parasitaemia level (P > 0.05). The findings of this study suggest the potential adoption of rapid ICT as a complementary epidemiology tool for malaria diagnosis among expectant women in areas of low parasitaemia and where microscopy is difficult to implement due to limited resources. The interventions on malaria control should also be geared towards control of malaria among all pregnant women irrespective of age and parity.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Malaria is a parasitic infection transmitted by mosquitoes and is one of the most devastating tropical infectious diseases in the world. Worldwide, around 3.4 billion people are at risk of malaria infection. In 2012 there were estimated 207 million cases of malaria and 627,000 cases of death were reported (WHO, 2013). Between the year 2000 and 2012, worldwide, malaria mortality rates have dropped by 42% across all age groups and by 48% among children under 5 years of age (WHO, 2013). The decline is due to employment of malaria control interventions by the World Health Organization through indoor residual spraying to control malaria vector, widespread use of long-lasting insecticide-treated mosquito nets (ITNs) and the use of artemisinin-combination therapy.

Globally, 125 million women are at risk of malaria infection every year in endemic areas of sub-Saharan Africa and 10,000 malaria related deaths have been reported due to maternal anaemia (Nosten et al., 2010; Dhiman et al., 2012). A study carried out recently between the year 2000 and 2011 in sub-Saharan Africa reported a malaria prevalence rate of 35.1% in West and Central Africa and 29.5% in East and Southern Africa among women attending antenatal clinics. Pregnant women are highly vulnerable to malaria infection due to lack of adequate immunity against variant surface proteins expressed by the parasites on the surface of the infected red blood cells. The infected erythrocytes sequester in the placenta and may be present throughout the whole pregnancy period (Cohee et al., 2014). Malaria during pregnancy has been greatly associated with maternal
anaemia, low birth weight, premature births and high infant mortality (Hartman et al., 2010). These effects are due to pathophysiological and haemolytic crisis affecting the mother (Kalilani-Phiri et al., 2013). Since 1920’s malaria epidemics have been reported in Western Kenya highlands usually leading to high levels of morbidity and mortality rates (Hay et al., 2002). Different studies have revealed that prevalence of malaria and parasitaemia level is higher among pregnant women of young maternal age and in their first or second pregnancy. This could be due to the fact that the first pregnancy may act as an additional physiological and metabolic stress to the mother. However, these findings vary depending on geographical area, malaria endemicity and transmission rates (Cot and Deloron, 2003; Duffy et al., 2006).

Accurate detection and prompt treatment is extremely essential in effective malaria control. However, microscopy which is the gold standard method for identifying malaria infection has several draw backs. It requires high proficiency to get accurate results since malaria parasites can be sequestered in the placenta; it is labour intensive and time consuming and thus not suitable in areas that report high cases of malaria and have limited resources (Harchut et al., 2013). Numerous molecular and immunological techniques have been developed as complimentary tests to support parasitological techniques but their sensitivity and accuracy in diagnosis of malaria among expectant women under different ecological areas have not been fully tested. The aim of this study was to compare the performance of the rapid immunochromatographic strip test with microscopy using polymerase chain reaction as a confirmatory test in diagnosis of malaria during pregnancy. The study also examined the effect of age and parity on
malaria prevalence and parasitaemia level among expectant women attending the antenatal clinic in Kericho District Hospital and Kipsitet Health Centre in Kericho County.

1.2 Statement of the problem

The gold standard method for malaria diagnosis is microscopy but numerous challenges are associated with performing this technique (Harchut et al., 2013). It is time consuming, labour intensive, require electricity to perform it and it is highly dependent on expertise hence more training is required to establish and sustain competence in performance (Harchut et al., 2013). According to Dondorp et al. (2011), this conventional technique is not an effective strategy in malaria diagnosis especially among the expectant women due to its low sensitivity. In addition, it does not detect all the parasites of Pf due to sequestration in the placenta during pregnancy (Goldring, 2014). These parasites have been associated with maternal anaemia and poor foetal outcomes in pregnant women thus increasing the risk of perinatal and maternal mortality (Kalilani-Phiri et al., 2013).

Therefore, there is need to evaluate the performance of rapid ICT as a complementary tool in diagnosis of malaria among expectant women in Kericho Highlands. Previous findings by Cot and Deloron (2003) and Duffy et al., 2006 also show that women in their young maternal age and in their first pregnancy are highly susceptible to malaria although these findings vary in different ecological zones. This also raised the need to determine
whether there is any effect of age on parity among expectant women in Kericho Highlands.

1.3 Justification of the study

Pregnant women are highly susceptible to malaria infection which contributes significantly to high cases of maternal and foetal mortality (Rogerson et al., 2000). Irrespective of the numerous challenges associated with conventional microscopy, it is widely used as the gold standard test in malaria diagnosis. The technique has low sensitivity and misses out on some of the malaria parasites in red blood cells circulating in the placenta during pregnancy (Goldring, 2014), this has led to underestimation of malaria prevalence in Kericho highlands. This draw back has led to evaluation of the performance of rapid immunochromatographic test in diagnosis of malaria since they may be a suitable complementary epidemiological tool for malaria diagnosis among the expectant women in low parasitaemia areas due to their high sensitivity and accuracy (Kyabainze et al., 2010). Confirmation of the suitability of rapid ICT in diagnosis of malaria among the expectant women will promote accurate detection of malaria parasite hence effective malaria control and reduced maternal and foetal mortality. According to Cot and Deloron (2003) and Duffy et al. (2006), women in their younger maternal ages and in their first pregnancy are more likely to be affected by malaria infection than their older counterparts and those in their second and subsequent pregnancies. Determination of the effects of age and parity on malaria prevalence and parasitaemia level in Kericho County will enable pinpoint which group of expectant women are more at risk hence intervention approaches can be tailored accordingly for effective malaria control.
1.4 Research questions

i) What is the sensitivity, specificity, positive and negative predictive values of the rapid immunochromatographic test?

ii) What is the effect of age and parity on malaria prevalence and parasitaemia level among expectant women?

1.5 Hypotheses

i) The performance of rapid immunochromatographic test is comparable to microscopy in diagnosis of malaria among expectant women.

ii) Age and parity have no significant effect on malaria prevalence and parasitaemia level among expectant women.

1.6 Objectives of the study

1.6.1 General objective

To determine the performance of rapid immunochromatographic test in diagnosis of malaria among expectant women and the influence of age and parity on malaria prevalence and parasitaemia level.

1.6.2 Specific objectives

i. To determine the sensitivity, specificity, positive and negative predictive values of the rapid immunochromatographic test in diagnosis of malaria among expectant women.

ii. To determine the effect of age and parity on malaria prevalence and parasitaemia level among expectant women.
1.7 Significance of the study

The findings of this study will shed light on reliability and suitability of rapid immunochromatographic strip test as a complementary epidemiological tool in diagnosis of malaria among pregnant women in Kericho Highlands and also promote its use in areas where use of light microscopy may have low accuracy or low detection levels. The findings on effect of age and parity on malaria prevalence and parasitaemia level will guide the government on which group of expectant women to target to not only reduce maternal and foetal mortality but also to effectively control malaria.
2.1 Life cycle of *Plasmodium* species in man

Malaria is a parasitic infection that is transmitted by female anopheline mosquito. *Plasmodium vivax, P. malariae, P. ovale* and *P. falciparum* are the commonly known causative agents of human malaria although recently *Plasmodium knowlesi* has also been incriminated as an aetiological agent of human malaria (Cox-Singh et al., 2008; Luchavez et al., 2008; McCutchan et al., 2008). *Plasmodium falciparum* is the major cause of severe malaria and has been associated with high fatality rate cases (Enato et al., 2008; Campos et al., 2012). Human host gets infected when infected female anopheles mosquito injects sporozoites into the dermis as it sucks blood. The sporozoites are carried in the liver where they multiply and differentiate into merozoites within the hepatocytes (Figure 2.1 below).
Figure 2.1: Life cycle of *Plasmodium* species (Jones and Good, 2006).

Merozoites released from the liver invade erythrocytes initiating blood stage of infection. Merozoites found within the erythrocytes undergo asexual reproduction maturing into ring stage then trophozoites and finally into schizont forms (Jones and Good, 2006). Schizonts rupture releasing merozoites (roughly 16–20 merozoites depending on *Plasmodium* spp), which then invade more red blood cells. Some of the parasites in the blood stage of infection differentiate into either female or male gametocytes which are then ingested by mosquito as it feed on blood meal. The female and male gametocytes
deposited in the mid gut of the mosquito undergo sexual reproduction forming more sporozoites (Aravind et al., 2003). The numerous stages involved in the life cycle of a malaria parasite within the human host enable it to present a diversity of antigens to the immune system. Majority of these antigens are polymorphic and undergo antigenic variation as a means of evading recognition by the immune system of the host (Miller et al., 2002). The ability of Pf to bind to endothelium during erythrocytic stage and to sequester in some organs such as the brain and placenta is what distinguishes it from other Plasmodium species (Menendez et al., 2000; Cox-Singh et al., 2008).

2.1.1 Pathological and immunological effects of *Plasmodium falciparum* infection

*Plasmodium falciparum* parasite evades the host immune system by expressing diverse antigens on surface of infected red blood cells. Variant surface protein *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is a member of var genes which are associated with antigenic variation and plays a primary role in host-parasite interaction. PfEMP1 is the immunodominant surface antigen which is involved in adhesion reactions leading to its sequestration as a means of avoiding destruction (Craig and Scherf, 2001). These antigens are the major cause of complicated malaria and have been adopted as major candidates in improvement of intervention approaches aimed at preventing parasite adhesion and disease protection. During pregnancy, *Plasmodium falciparum* cytoadhere to the syncytiotrophoblast which expresses numerous receptors of chondroitin sulfate A (CSA). The Duffy binding-like (DBL)--γ3 domain of PfEMP1CSA to the CSA receptor is responsible for sequestration of the infected RBCs to the intervillous space of placenta leading to maternal morbidity, spontaneous abortion, low birth weight
and premature delivery especially among African women in their first pregnancies (Scherf et al., 2001).

Malaria parasite-infected red blood cells from non-pregnant women do not express PfEMP1 antigen and do not bind to CSA receptor expressed on syncytiotrophoblast (Scherf et al., 2001; Rogerson et al., 2007). According to Rogerson et al. (2007), women can only develop immunity to placental parasites after being pregnant and the first pregnancy allows them to acquire immunity against CSA-parasite binding receptor hence are protected from malaria infection in the subsequent pregnancies. Besides CSA receptor, some PfEMP1 members bind in vitro to other receptors including CD36, thrombospondin and intercellular adhesion molecule 1 (ICAM-1). Additionally, erythrocytes infected by Plasmodium falciparum bind to platelet endothelial cell adhesion molecule 1 (PECAM1), vascular cell adhesion molecule 1 (VCAM-1) and E selectin although the binding properties of these receptors to PfEMP1 is yet to be investigated (Reeder et al., 1999).

2.2 Epidemiology of malaria in pregnant women

Pregnant women are highly vulnerable to malaria and mortality rate arising from severe malaria is approximately 50% (WHO, 2012). According to Desai et al. (2007), malaria has affected roughly 25% of the expectant women in malaria endemic areas of Sub-Saharan Africa, and the primigravidae, women in their young maternal age (adolescent)
and those with HIV co-infection are the most affected. However, women in their second trimester are more affected by malaria hence efforts on prevention measures and treatment should be geared towards this group. Depletion of cell mediated immunity (CMI) and sequestration of the parasitized red blood cells in the placenta is the major cause of complications during pregnancy (Desai et al., 2007). According to Monif and Baker (2004), women with high levels of acquired immunity against malaria have increased foetal complications due to accumulation of numerous parasites in the placenta which cause milder or no maternal symptoms. However, several studies show that the level of acquired immunity influencing malaria prevalence and parasitaemia level among pregnant women vary depending on physiological factors such as maternal age and parity, ecological settings and malaria endemicity (Desai et al., 2007).

2.3 Pathophysiological and immunological effects of malaria on the placenta

The placenta is an important organ that provides sustenance to the foetus during the gestation period. It is also an immuno-privileged site highly preferred by Plasmodium falciparum since it helps in evading destruction by the immune system. At term, the structure of placenta is circular with a thickness of 2.5 cm and diameter of 20 cm. The placenta has a chorionic plate and villi at the foetal portion and a peripheral trophoblastic shell surrounding the intervillous space of maternal tissue (Fried et al., 2006).

Sequestration of Plasmodium falciparum expressing PfEMP-1 antigen in the intervillous space of placenta is the major cause of malaria during pregnancy (Hviid, 2010). Sequestration is the adherence of plasmodium falciparum usually trophozoites and
schizonts (late developmental stages) to the endothelium of capillaries and venules of placenta. Proliferation of malaria parasite and subsequent activation of endothelial cells leads to production of adhesion molecules which promote the infected erythrocytes to sequester in the placenta (Figure 2.2). The syncytiotrophoblast of placenta expresses numerous receptors of chondroitin sulfate A (CSA) which is responsible for sequestration. Sequestered parasites sensitize the inflammatory cells to release chemical mediators which cause alterations to the placenta (Rogerson et al., 2007). Leucocytes produce non-chemotactic cytokines which thicken the basement membrane of trophoblast thus blocking oxygen flow and nutrient transport. Intervillous macrophages reduce maternal blood flow hence affecting exchanges involved between the mother and the foetus (Hartman et al., 2010). This leads to adverse outcomes such as maternal anemia, preterm birth, low birth weight and stillbirth. Low birth weight and maternal anaemia are the major serious effects, with the latter being the primary cause of neonatal and infant morbidity and mortality (Desai et al., 2007; Hartman et al., 2010). Therefore, there is need for accurate detection of the malaria parasite in order to reduce maternal and foetal mortality.
2.4 Immunological aspects related to parity and age in women during pregnancy

According to Cot and Deloron (2003), primiparous women are highly affected by malaria infection compared to multiparous women. The decreased susceptibility to malaria during pregnancy with increased parity is associated with the development of antibodies against *P. falciparum*-1 variant surface antigens expressed by parasitized red blood cells (Duffy *et al.*, 2006).

A study conducted by Duffy and Fried (2005) revealed that malaria antibodies developed in multigravid mothers prevents adhesion of malaria parasites to CSA receptors expressed by placenta (syncytiotrophoblast) in subsequent pregnancies. Some studies
have also reported high susceptibility to malaria among women in their younger maternal ages than in older women because they have not developed adequate immunity to malaria infection (Johnson et al., 2004; Drakeley et al., 2005). A study conducted by Ali et al. (2011) among expectant women in Sudan showed that these women are highly susceptible to *Plasmodium falciparum* infection and prevalence rate is high regardless of parity and age. Findings from a study in Cameroon also revealed that prevalence of malaria was influenced by age and that younger women and those in their first pregnancy were more susceptible (Tako et al., 2005; Oliver et al., 2014). Development of antibodies that block the binding of malaria parasites to CSA receptors may be very important in younger women than in older women who may not only depend on anticytoadherent antibodies but also adequate immunity acquired through recurrent exposure to malaria infection (Tako et al., 2005). In non-endemic areas and epidemic zones, malaria prevalence is high in all groups of people, though higher among children below the age of 5 years and expectant women because they have not developed immunity from previous infection (Johnson et al., 2004). Age and parity have been found to influence malaria prevalence and parasitaemia level among the expectant women but the findings vary depending on geographical area, transmission rate and endemicity (Johnson et al., 2004; Tako et al., 2005).

### 2.5 Immunity to malaria

Populations that are continuously exposed to malaria parasite especially in malaria endemic areas develop natural defense mechanisms against the infection (Tako et al., 2005). In areas where malaria transmission is stable, the infection mostly affects children
in their early years of life and symptoms are severe for children under the age of 5 years. Severity of disease and parasite density decrease as immunity develops and the immune response acquired is strain specific.

Following infection by malaria, antibodies bind to parasitized erythrocytes in order to enable macrophages and other cells of the immune system to ingest the diverse antigens produced by Pf. Complement proteins are very useful in destroying malaria parasites and averting infection of new erythrocytes and their effectiveness is promoted by antibodies (Rogerson et al., 2007). The T cells recognize and bind on macrophages expressing malaria parasite antigens on their surfaces. They become activated to release cytokines which kills the parasite and activates B cells to produce antibodies which enable the macrophage to ingest more parasitized cells which are either killed by activated macrophages or by T cells through release of cytokines. In case the infected erythrocytes rupture, they release numerous parasites into the blood stream. As a result, macrophages respond by releasing tumor necrosis factor, a cytokine that destroys the parasites. However, the cytokine causes malaria fever which has been associated with premature labor during pregnancy (Hartman et al., 2010).

2.5.1 Clinical symptoms of malaria

The earliest malaria symptoms include body weakness, malaria fever, chills, headache, dizziness, myalgia, abdominal pain, nausea, anorexia, diarrhoea and vomiting (Pouniotis et al., 2004). These symptoms are variable and non-specific and considerably overlap with common febrile illnesses and other common bacterial and viral diseases. This nature
of similarity between malaria symptoms and symptoms of other febrile infections affects the diagnostic specificity and promotes unnecessary use of anti-malarials. This also affects the care and management of patients with fever that’s not necessarily caused by malaria especially in areas endemic for malaria (Pouniotis et al., 2004).

2.6 Malaria control

The WHO Global Malaria Programme recommends the vector control (use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of houses), chemotherapy and diagnostic testing and appropriate treatment as intervention strategies geared towards effective malaria control and elimination (WHO, 2011).

2.6.1 Vector control

World Health Organization recommends wide coverage and accessibility to insecticide treated nets especially in malaria endemic areas for effective malaria vector control (WHO, 2011). Both conventionally treated nets and long lasting treated nets are made of a netting material which is treated to kill the mosquito when it comes into contact with it. Conventionally treated nets are retreated annually or after washing three times with an insecticide that is recommended by WHO to promote the effectiveness of the insecticide. Long lasting insecticide treated nets are treated in the factory and do not require retreatment to promote the effectiveness of insecticide and thus can be used for at least 20 washes or for 3 years (WHO, 2011). Full coverage is emphasized among the expectant women and younger children especially under the age of 5 years since they are very susceptible to malaria infection. Children clinics and antenatal care clinics for pregnant
mothers have promoted the distribution of ITNs to the target groups thus enhancing wide coverage (Gamble et al., 2006; WHO, 2011). In areas where malaria transmission is low or unstable, ITNs should be distributed to all people to promote total protection although malaria endemic areas and those at risk of malaria outbreak should be given the first priority (Hawley et al., 2003).

Indoor Residual Spraying (IRS) is an effective intervention strategy that uses long lasting and effective insecticides for controlling malaria vectors found in houses and animal structures. As the vector rest on the surface of the walls and ceiling, they get into contact with the insecticide which reduces their lifespan and ultimately reduces vector density. This intervention targets both endophillic mosquitoes (resting on surfaces) and endophagic mosquitoes (those searching for a blood meal in human habitats). Decreased life span and density of female anopheline mosquito through IRS effectively contributes to reduction of malaria transmission (Lluberas, 2013; WHO, 2013).

2.6.2 Chemotherapy
Intermittent Preventive Treatment in pregnancy is a malaria control intervention strategy recommended among expectant women in areas that are highly burdened by malaria. Treatment is done using sulphadoxine-pyrimethamine (SP) which is effective in averting any possible complication of malaria to both the mother and the foetus. At least two doses are administered to the pregnant women in their 2nd and 3rd trimesters of gestation during antenatal care visits (WHO, 2012).
2.7 Diagnosis of malaria

Presently, limited methods are available for malaria diagnosis. Conventional methods include empirical diagnosis and microscopy. Empirical diagnosis relies on patient’s clinical history and physical examination (WHO, 2011). Polymerase chain reaction is a molecular technique that is used in malaria diagnosis but this assay is limited by complex methodologies and high cost (Luchavez et al., 2008). Immunological techniques such as Malaria Rapid Diagnostic Test have been developed and marketed and could be most effective in accurate malaria detection (Murray et al., 2008).

2.7.1 Clinical diagnosis

Clinical diagnosis relies on signs and or symptoms and patient’s history of the disease. The presence of fever is a major indicator of malaria infection. In many malaria endemic areas with limited diagnostic capacity, treatment of patients with a febrile illness using anti-malarials has been reported. WHO disapproves this approach especially in areas where malaria diagnostic test is available due to numerous pitfalls associated with it (WHO, 2013). First, malaria symptoms are similar to symptoms of febrile illnesses and fever alone is very non-specific to be relied on in making any diagnosis. Secondly, co-infections occur and treating one of the infections leaves the other untreated. Thirdly, patients may have malaria parasitemia which may not necessarily be the cause of fever thus treating these patients with antimalarials results in using antimalarials for other illnesses that are not malaria (Bell et al., 2006). Therefore, clinical diagnosis should be combined with parasite detection to enhance accuracy in malaria diagnosis (Kyabayanze et al., 2010).
According to World Health Organization, effective malaria diagnosis is important in order to enhance effective treatment by limiting treatment with antimalarial to only patients who have malaria infection (WHO, 2011). Clinicians have also reported to have advised on treating pregnant women with antimalarials whose smears test negative claiming that microscopy misses some submicroscopic parasites due to sequestration in the placenta (Adam, 2008).

2.7.2 Parasitological diagnosis

Microscopy is the recommended test by WHO for detecting malaria parasitemia as well as the reference test against which all other tests are compared (Hawkes et al., 2007; WHO, 2011). This technique also allows species determination, estimation of parasite density (parasitaemia level) and differentiation of different stages of malaria parasite (McKenzie et al., 2003). Microscopy involves preparation of thick and thin blood films and examination under a microscope. The patient finger is first cleaned with a cotton swab containing 70% ethyl alcohol, allowed to dry then pricked using a sterile lancet. Two drops of blood are collected on a slide each for thick and thin smear. The thin smear is fixed with absolute methanol while thick smear is allowed to air dry without any fixation. The slides are then stained in a 10% giemsa for a period of 10 minutes. This is then followed by washing in distilled water and allowed to dry in air in a vertical position. Thin film should not be washed excessively to avoid decolorizing the film. After drying, the slides are examined under a high power magnification (100 X- oil immersion) (Chotivanich et al., 2006). Giemsa stain is the gold standard stain recommended for staining malaria slides since it reveals distinct features of malaria
parasite such as; maurers cleft of *Plasmodium falciparum* and Schuffner dots for *Plasmodium vivax* on thin film. Thick film is very sensitive in detection of malaria parasite while thin film allows species determination (Reyburn *et al.*, 2007).

Although microscopy is the acceptable and widely used technique in malaria diagnosis, numerous challenges are associated with it. The procedures involved in staining are time consuming, labour intensive and the technique cannot be used in remote areas where electricity is unavailable or unreliable. Accurate detection of malaria parasite at low parasite density, differentiation of species and different stages of malaria parasite require experienced microscopists. Although expert microscopists can identify parasites as low as 5 parasites/µl, while average microscopist identifies 50-100 parasites/µl, low level microscopists can underestimate malaria parasites at low parasitaemia level giving false negative results (Payne, 1998). A lot of training and continuous assessment of microscopists on accurate detection of malaria parasites, species differentiation and estimation of parasitaemia level can promote competence in performance and reproducibility of results by microscopy. However, this has not been possible in malaria endemic areas of developing countries due to limited resources to facilitate trainings hence reducing the reliability of microscopy in malaria diagnosis (Reyburn *et al.*, 2007). These drawbacks limit the use of microscopy in diagnosis of malaria hence promoting evaluation of rapid immunochromatographic test which is associated with high sensitivity and accuracy in malaria diagnosis.
2.7.3 Quantitative buffy coat technique

The Quantitative buffy Coat is a simple and a reliable technique that was developed to improve diagnosis of malaria by microscopy (Clendennen et al., 1995). It utilizes epi-fluorescent microscopy to detect malaria parasite DNA which is stained with fluorescent dyes such as acridine orange. Hematocrit tubes are used in staining and the parasite is easily identified since the nuclei fluoresces bright green, while cytoplasm assumes yellow-orange colour. Although this technique is simpler to use than conventional light microscopy, it’s more costly and requires specialized instruments. It is also not useful in differentiation of species and calculating parasite density (Bhandari et al., 2008).

2.7.4 Molecular diagnostic methods

Molecular diagnostic methods display high performance based on sensitivity and specificity. Recent developments in molecular biological technologies, include; Polymerase chain reaction (Chotivanich et al., 2006), loop-mediated isothermal amplification (LAMP) (Poon et al., 2006) and flow cytometric (FCM) assay techniques (Wongchotigul et al., 2004). Advantages and limitations of each of the above molecular techniques are described below.

2.7.4.1 Polymerase chain reaction

Polymerase Chain Reaction is a molecular technique developed recently for malaria diagnosis. PCR principle involves amplification of a segment of DNA (Deoxyribonucleic acid) to generate numerous copies which may range from thousands to million copies. This technique has high specificity and sensitivity in detecting malaria parasites of low
density and can detect a mixed infection. This technique has also been used to confirm whether an infection is caused by malaria, detect cases of drug resistance and follow up on treatment response (Chotivanich et al., 2006). Polymerase chain reaction can identify as low as 1-5 parasites/µl of blood compared microscopy and RDTs which detect between 50-100 parasites/µl. Recent advancement in molecular biology has also led to development of real time PCR, reverse transcription PCR and nested PCR which are highly modified and reliable in malaria parasite detection. Polymerase chain reaction has also been used as a second-line techniques to confirm the results of conventional technique when patients present with malaria symptoms (Mlambo et al., 2008). The technique is also applicable in species determination and quantification of malaria parasites. Recently, PCR method has been used in identification of Plasmodium knowlesi, a newly discovered malaria parasite (Cox-Singh et al., 2008; Luchavez et al., 2008). Although PCR has high specificity and sensitivity, its use is limited by expensive machine which requires quality control and maintenance, and need for trained technicians since the methodologies involved are complicated. As a result, PCR is not routinely implemented in developing countries with limited resources (Mens et al., 2008).

2.7.4.2 Loop-mediated isothermal amplification (LAMP) technique

The LAMP technique is a gene amplification method which amplifies DNA with high specificity and efficiency under isothermal conditions. It is based on the use of a set of 4-6 specially designed set of primers which span 6-8 distinct sequences on the target DNA. The technique is simple, rapid and an inexpensive molecular tool used in malaria diagnosis and detects the conserved 18S ribosome RNA gene of P. falciparum (Poon et
al., 2006). Other studies have shown that LAMP can accurately detect the four malaria parasites species (*P.* *vivax*, *P.* *malariae*, *P.* *ovale* and *P.* *falciparum*) (Han *et al*., 2013). Although Lamp has high specificity and sensitivity and is also less costly than PCR its use is limited by reagents which must be stored under cold conditions (Erdman and Kain, 2008).

### 2.7.4.3 Flow cytometer (FCM) assay

Studies have reported the use of flow cytometer in diagnosis of unsuspected cases of malaria. The technique detects hemozoin produced by malaria parasites when they digest haemoglobin of the host. Hemozoin is identified when laser light is depolarized as cells flow through the channel (Wongchotigul *et al*., 2004). Flow cytometer has a specificity of 82-97% and sensitivity of 49-98% in diagnosis of malaria (Padial *et al*. 2005). This technique is limited by expensive equipment, laborious procedure, time consuming and high cases of false negatives resulting from bacterial and viral infections.

### 2.7.5 Immunological malaria diagnostic tests

Reliable immunological malaria diagnostic methods include, Immunofluorescence antibody testing (IFA) (She *et al*., 2007); Enzyme linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA) (Park *et al*., 2008), Serological tests (Reesing, 2005; She *et al*., 2007), Latex agglutination assay (Polpanich *et al*., 2007) and malaria rapid diagnostic devices (Murray *et al*., 2008).
2.7.5.1 Serological tests

Serological test is based on detection of *Plasmodium* antibodies produced against malaria parasites during asexual stages of reproduction. Immunofluorescence antibody testing (IFA) has been widely accepted as a gold standard in serological diagnosis of malaria due to its high sensitivity and specificity (Doderer et al., 2007). Immunofluorescence antibody testing is used in blood transfusion centres to screen donors for *Plasmodium* antibodies to prevent transfusion of blood that has malaria infection. Although IFA is highly reliable, it is limited by procedures which are laborious, time consuming and use of fluorescent microscopy requires experienced technicians (She et al., 2007; Robertson, 2008).

2.7.5.1.1 Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assay is a rapid and a highly sensitive test that detects IgG and IgM antibodies produced by malaria parasite. Specific antibodies in the blood sample bind to antigens immobilized on a 96-well ELISA plate. A total extract from *Plasmodium falciparum* cultivation enriched with recombinant antigens from *P. vivax* is used as an antigen in detection of antibodies against *P. vivax* and *P. falciparum*. ELISA has also allowed detection of antibodies against *P. malariae* and *P. ovale*. The unbound material is removed during washing and peroxidase (HRP)-linked antibodies directed against human IgG and IgM are used during the incubation. Excess peroxidase (HRP)-linked antibodies are washed off and final incubation with HRP substrate solution based on tetramethylbenzidine (TMB) and hydrogen peroxide leads to a blue coloration which is correlated to the amount of antibodies in the patient’s specimen. Addition of dilute
sulphuric acid halts the enzymatic reaction and the absorbance values are measured at 450 nm (Park et al., 2008).

2.7.5.2 Rapid diagnostic methods

Rapid diagnostic test is a simple to use rapid technology with high sensitivity and specificity and is designed to detect *Plasmodium* specific antigens. Labeled antibodies bound to the well of test kit bind to parasite antigen in the patient blood sample, move through capillary action (immunochromatography) and are captured by antibodies bound at the test line producing a colored band. They require little expertise to perform and give easily interpretable and reproducible results; reagents do not require refrigeration and can be used in areas with no electricity and minimal or no laboratory infrastructure (Murray et al., 2008). World Health Organization recommends the use of this rapid diagnostic technology in areas where microscopy and other malaria diagnostic techniques cannot be implemented (WHO, 2013).

Different types of malaria rapid diagnostic kits have been developed and the type depends on the type of monoclonal antibodies or immunoglobulin (IgG or IgM) used to capture the parasite antigen. Currently available RDTs approved by World Health Organization include; SD BIOLINE malaria Ag *Pf* / Pan manufactured by Standard Diagnostic Inc.; Care start malaria HRP2 (*Pf*) by Access Bio Inc and Paracheck *Pf* rapid test for *Pf* malaria by Orchid Biomedical system (WHO, 2013).
2.7.5.2.1 Malaria diagnosis using RDTs

The available malaria rapid diagnostic tests detect *Plasmodium falciparum* histidine-rich protein 2 (HRP-II) antigen, *Plasmodium spp* lactose dehydrogenase (pLDH) and aldolase enzyme. *Plasmodium falciparum* histidine-rich protein 2 (HRP-II) contain repeat regions of histidine rich amino acids which are only produced by *Plasmodium falciparum* parasites. Therefore, RDTs containing monoclonal antibodies against HRP-II will only detect *P. falciparum* infection. HRP-II allows detection of *P. falciparum* during blood stage of infection and gametocyte stage. According to Goldring (2004), as *P. falciparum* develops from ring stage to trophozoites, they disappear from the peripheral circulation and sequester in organs including the brain and placenta hence cannot be detected using microscopy. However, RDTs specific for HRP-II can detect HRP-II protein produced by *P. falciparum* parasites sequestered in the organs. One of the limitations of HRP2- RDTs is that polymorphism may occur on HRP2 antigen gene and any deletions of the HRP2 gene may lead to false negative results. Pro-zone effects resulting from excess antibodies or antigen may also lead to false negative results using RDTs based on HRP-II (Gillet *et al.*, 2009; Luchavez *et al.*, 2011).

Lactose dehydrogenase (LDH) is designed to detect specific species of *Plasmodium* or all species of *Plasmodium* (pan specific) (Murray *et al.*, 2008). LDH enzyme is produced by the four species of *Plasmodium* (*P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*) in both sexual and asexual stages of reproduction. All the sequences of LDH expressed by *Plasmodium* species share similar epitopes, thus if RDTs based on pLDH with a monoclonal antibody targeting a common epitope is used, it detects a mixed infection
containing all the four *Plasmodium* species (Mayxay *et al.*, 2004). RDTs targeting pLDH are preferred over HRP-II since LDH antigen does not undergo antigenic variation and does not reveal any prozone effect. In addition, LDH is not detected after parasite clearance in the blood stream unlike HRP-II hence has been used in drug-sensitivity testing since it only detects viable parasites (Fogg *et al.*, 2008; Gerstl *et al.*, 2010).

Aldolase is an enzyme that is produced by all malaria species thus cannot be used to detect a specific *Plasmodium* species. Aldolase has been used in connection with RDT targeting PfHRP-II to detect *Plasmodium falciparum* and non-*Plasmodium falciparum* species (Richter *et al.*, 2004). RDTs based on combination of pLDH, PfHRP2 and aldolase can effectively be used in diagnosis of malaria due to the capacity to detect mixed infections (Maltha *et al.*, 2013).

### 2.7.5.2.2 Mechanism of action of malaria rapid diagnostic tests

Malaria rapid diagnostic kits are designed to detect antigens produced by malaria parasites. A visible band forms on the test line in case the parasite antigen is detected in the blood sample, indicating a positive result while a visible band on the control line indicates a negative result. The blood is applied on the well where it mixes with the lysing agent which ruptures the red blood cells to release parasite protein (Figure 2.3 below). The parasite protein is captured by dye labeled antibodies on the well and the mixture is carried by capillary action through the nitrocellulose strip. The mixture of parasite protein and dye labeled antibodies is captured by the antibodies bound to the test line. These antibodies are specific to the parasite antigen and hence bind to different
epitopes of the antigen resulting to formation of a colored band (Positive result). Antibodies bound to control line only bind to the remaining epitopes of dye labeled antibodies which were not bound by parasite antigen in the blood sample (Luchavez et al., 2011; WHO, 2012).

Figure 2.3: Mechanism of action of malaria rapid diagnostic tests (Luchavez et al., 2011).

2.8 Sensitivity, specificity and predictive values of a diagnostic test

The performance of a diagnostic test is determined by sensitivity, specificity, positive predictive value and negative predictive value. A 2x2 table allows the calculations of sensitivity, specificity and predictive values as described by Loong (2003). Sensitivity is
the ability of a test to correctly identify those with the disease (true positive rate). A test with 100% sensitivity will recognize all patients with the disease by testing positive. Sensitivity is calculated by dividing the number of (true positives) with the sum total number of (true positive and false negative) On the other hand, specificity is the ability of the test to correctly identify those without the disease (true negative rate). A test with 100% specificity will read negative, and accurately exclude disease from all healthy patients. A positive result signifies a high probability of the presence of disease. Specificity is calculated by dividing the number of (true negatives) with the sum total number of (true negative and false positive), (Rumsey, 2003; WHO, 2012).

The positive predictive value (PPV) gives the proportion of people who tested positive for the infection and are truly infected. Negative predictive value (NPV) gives the proportion of people whose test results are negative and are truly uninfected. The predictive value of a test under evaluation is dependent on sensitivity and specificity of the test and prevalence of disease for which the test will be used to diagnose. The specificity, sensitivity and predictive values of a test under evaluation may also vary in different ecological settings (Bland, 2000; WHO, 2012).

2.9 Malaria diagnosis in pregnant women

The literature review presented above clearly identifies pitfalls or limitations in diagnosis of malaria during pregnancy. Although, PCR-based techniques have proven to be one of the most specific and sensitive molecular method in diagnosis of malaria their use is limited by complex methodologies and high cost hence not suitable in routine diagnosis
of malaria and in remote rural areas (Mens et al., 2008). Microscopy has been recommended as the gold standard in diagnosis of malaria but this technique seems to be limited in diagnosis of malaria due to its low sensitivity and specificity (Harchut et al., 2013). This conventional technique may miss out on some of the malaria parasites due to sequestration of parasitized red blood cells in the placenta during pregnancy and may not be suitable in areas with low malaria parasite density. The recently developed rapid immunochromatographic test may overcome the limitations of conventional and PCR-based techniques because the technique has high sensitivity and specificity, it is easily available, cost effective, give reproducible results and can be used in remote rural areas without electricity. The ability of rapid ICT to detect malaria parasite sequestered in the placenta (Goldring, 2004) which cannot be detected using microscopy may make it a suitable alternative in future diagnosis of malaria among expectant women. The objective of the present study is therefore to evaluate the performance of rapid ICT in diagnosis of malaria among expectant women to determine if they can be used to complement microscopy in malaria diagnosis during pregnancy hence promoting effective malaria control.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

This study was conducted in Kericho County (0.3667° S, 35.3000° E) which is located in the highlands of the Rift valley. The study sites were Kericho District hospital and Kipsitet health centre (Appendix I) and were selected through purposive sampling method. These centers have limited resources, rely on microscopy in diagnosis of malaria and have an antenatal care (ANC) clinic which record high antenatal clinic visit. The rainfall patterns are bimodal with long rainy season between April and June and short rains from November to December.

Most of Kericho County is an area with unstable malaria transmission that peaks during the long (March- May) and short (October-December) rainy seasons. The two health centres have an antenatal clinic that offers maternal care services to expectant women in the surrounding area and outside the locality at least two days in a week. It also has limited resources and relies on microscopy in diagnosis of malaria among expectant women attending the antenatal clinic. The vegetation is bushy with temperatures ranging between 25-32°C which may favor vector breeding thus increasing malaria transmission. The main economic activity in the county is cash crop farming main crops being tea, maize, and sugarcane farming. There is also livestock keeping in the County.

3.2 Study design

The study involved all expectant women attending the antenatal clinic during their routine visit. The women were recruited as they visited the laboratory for malaria screening. After informed consent, a brief structured questionnaire was used to collect
information on parity and age. Five milliliters of blood were collected from participants and put in EDTA coated bottles for malaria diagnosis using microscopy, rapid ICT and molecular analysis using polymerase chain reaction (PCR). Microscopy and rapid ICT were done on site during blood collection while blood for molecular analysis was stored in filter papers and analyzed at Kenya Medical Research Institute located in Nairobi at the end of the blood collection exercise. The expectant women were treated based on the results of microscopy and those who tested positive for malaria infection received prompt treatment.

### 3.3 Sample Size

The sample size estimation was as per the WHO/TDR (2010) formula as follows:

\[ n \geq (1.96)^2 p(1-p) \]

\[ x^2 \]

Where:

- \( n \) is the sample size,
- \( p \) is the prevalence of malaria expressed as a proportion, and
- \( x \) is the level of significance or \( \alpha \)

\[ n = (1.96)^2 \times 0.05(1-0.05) = 73 \]

With assumed malaria prevalence of 5% (Kenya western highlands) and with a Confidence Interval of 95%, a sample size of 73 would be sufficient for the study though a total of 314 expectant mothers were recruited for the study in order to acquire an adequate number of positive samples for analysis.
3.4 Inclusion criteria

The study included all pregnant women aged 18 years and above attending antenatal care clinic who were referred to the laboratory by the clinician for the malaria test, and were not actively on antimalarial drugs. Only those who willingly signed an informed consent form participated in the study.

3.5 Exclusion criteria

All pregnant women not referred to the laboratory by the clinician for malaria diagnosis and those who were actively on antimalarial drugs were excluded from the study. Those who declined to consent and those aged below 18 years were also excluded from the study.

3.6 Blood collection and screening for malaria

Five milliliters of venous blood was collected from participants and put in EDTA coated bottles. The blood samples were then analyzed for presence or absence of malaria parasite using microscopy, rapid immunochromatographic test and PCR. Microscopy was also used to estimate parasitaemia level.

3.6.1 Determination of parasitaemia level by microscopy

Thick blood films were prepared by placing a drop of blood on a slide, spread in a circular motion and allowed to air dry. The slides were then stained for 10 minutes using 10% Giemsa stain, washed in distilled water and allowed to dry in a vertical position. The slides were evaluated using light microscopy (100 × oil immersion lenses) for malaria by two independent microscopists and the results were double blinded. Discrepancy in
results between the two microscopists was resolved by re-reading of the slides by a third microscopist before the final results were determined. Parasitaemia level was calculated by counting malaria parasites found in red blood cell in 200 leukocytes and multiplying the resulting value by 40 assuming that each microliter of blood contain 8,000 leukocytes as described by WHO (2012). Any discrepancy in parasite count readings was resolved by computing the mean of the parasitaemia reading by the two microscopists. If no parasite was recorded after counting 200 leucocytes, a slide was then considered as negative.

3.6.2 Rapid immunochromatographic test

The Rapid immunochromatographic test kit used in this study was SD Bioline 05FK60 One Step Malaria Antigen P. falciparum / Pan-test (Standard Diagnostic Inc.) and procedure for testing was done as described by the manufacturer. Briefly, using a capillary tube, 5 µL of whole blood was drawn from the EDTA bottle and transferred into a round sample well followed by 4 drops of assay diluent which were placed into a square well. The test results were read after 15 minutes. The test was considered positive if a band was formed at test line (PAN and Pf line) and negative if the band formed at the control line only (Figure 3.1).
3.6.3 Polymerase chain reaction assay

Three drops of blood were spotted directly on 3mm Whatmans filter paper at different points as described by Hsiang et al. (2010), allowed to air dry, then placed individually in plastic bags and stored at -20°C with a silica gel desiccant to prevent dampness for DNA extraction. Extraction of Parasite DNA from filter papers was performed using Chelex-100 method as described by Plowe et al. (1998). The piece of filter paper was transferred to a sterile 1.5 ml microfuge tube containing 1 ml of 0.5% saponin in 1X phosphate buffered saline (PBS) and incubated overnight at 4°C. The brown solution was discarded and replaced with 1 ml phosphate buffered saline and incubated at 4°C for 30 minutes. The brown solution was also discarded and 50 μl of stock 20% chelex solution added to 150 μl of DNase-free water in a 0.5 ml microfuge tube containing the filter paper. The component in the tube was heated at 100°C for 2 minutes followed by vortexing for 10 minutes. The tube was then centrifuged at 10 000 x g for 2 minutes and then the
supernatant transferred to another fresh tube and centrifuged for another 2 minutes. The final supernatant was transferred into another fresh tube to be used as a DNA template. The amplification of K-13 propeller gene was done on MJ Thermocycler™ PCR machine.

The PCR reaction involved outer and nested reaction in order to enhance specificity. One microlitre of DNA extracted through Chelex method was used as a template in outer PCR amplification and was added into a reaction mixture of 29 µL containing 3 µL 10 X PCR buffer, 3.6 µL 25 mM Magnesium Chloride, 0.3 µL 20 mM dNTPs, 0.3 µL of each 10 µM primer (K13-1 and K13-4), 1.2 µL Taq DNA polymerase (5 u/ µL) and 20 µL of double distilled water, making up a total volume of 30 µL (Appendix 11). The K13-propeller domain was amplified using, K13-1 5’-CGGAGTGACCAAATCTGGGA-3’ and K13-4 5’-GGGAATCTGGTGGTAACAGC-3’ as forward and reverse primers respectively. Thermocycling conditions were 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 90 seconds, 72°C for 90 seconds and a final extension of 72°C for 10 minutes. Two microlitres of outer PCR product was used in nested PCR amplification and was added into a reaction mixture of 28 µL containing 3 µL 10 X PCR buffer, 3.6 µL 25 mM Magnesium Chloride, 0.3 µL 20 mM dNTPs, 0.3 µL of each 10 µM primer (K13-2 and K13-3), 1.2 µL Taq DNA polymerase (5 u/ µL) and 19.3 µL double distilled water thus making up a total volume of 30 µL (Appendix III). The forward and reverse primer sequences used for amplification of K13 propeller gene in nested PCR were K13-2 5’-GCCAAGCTGCCATTCATTTG-3’ and K13-3 5’ GCCTTGTGGAAAAGAAGCAGA-3’ respectively. The thermocycling conditions for
nested PCR were similar to outer PCR. For each run, DNA extracted from laboratory-adapted *Plasmodium falciparum* was used as positive control while water was used as negative control. Analysis of PCR products was done using 2% agarose gel electrophoresis and visualization done under UV light on a transilluminator using gel documentation system (VilbertLourmat).

3.6.4 Calculation of sensitivity, specificity and predictive values

McNemar’s 2×2 contingency table (Table 3.1) was used to calculate the sensitivity, specificity, positive and negative predictive values of rapid ICT (test under evaluation) using microscopy as the gold standard and polymerase chain reaction as a confirmatory test. The number of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) were calculated. Sensitivity was calculated as TP/ (TP + FN). Specificity was calculated as TN/ (TN + FP). The positive predictive value (PPV) was calculated as TP/(TP + FP) and negative predictive value (NPV) was calculated as TN/ (FN + TN). Accuracy of the test, defined as proportion of all tests that gave a correct result, was calculated as (TP + TN)/ number of all tests.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>GOLD STANDARD TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST UNDER EVALUATION</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>A</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>C</td>
</tr>
<tr>
<td>TOTAL</td>
<td>a + c</td>
</tr>
</tbody>
</table>

Table 3.1: McNemar’s 2×2 table
Where;

\( a \) = True positives; \( b \) = False positives; \( c \) = False negatives and \( d \) = True negatives

**Sensitivity** = \( \frac{True \ positives}{(True \ positives + False \ negatives)} \times 100 \); **Specificity** = \( \frac{True \ negatives}{(False \ positives + True \ negatives)} \times 100 \); **Positive Predictive Value** = \( \frac{True \ positives}{(True \ positives + False \ positives)} \times 100 \) and **Negative Predictive Value** = \( \frac{True \ negatives}{(False \ negatives + True \ negatives)} \times 100 \)

### 3.7 Collection of demographic data

The data on age and parity of the mothers participating in the study was collected in a brief structured questionnaire. The age of the mothers ranged from 18 years and above. Both primiparous and multiparous women were included in the study regardless of stage of pregnancy. Multiparous women included all women who had delivered at least once while primiparous women had not delivered before (were in their first pregnancy).

### 3.8 Data analysis

The coded data was entered on MS-Excel and imported into IBM SPSS Statistics v 22.0 (IBM Corp., Armonk, NY) for analysis. Assessment of the agreement between rapid ICT, microscopy, microscopy and PCR was compared using Cohen Kappa Index. Chi-square analysis was used to assess whether parity has any significant effect on malaria prevalence. The effect of parity and age on parasitaemia was determined using Odds Ratio tests. Binary logistic regression was used to assess whether age and parity are predictors of malaria prevalence.
3.9 Ethical clearance

Approval of the study was obtained from Ministry of Medical Services in charge of Kericho County and from Moi Teaching and Referral Hospital/Moi University IREC. Written/oral informed consent was obtained before inclusion in the study. Only those who consented were allowed to participate in the study.
CHAPTER FOUR: RESULTS

4.1 Descriptive statistics of study participants

4.1.1 Age distribution

The present study enrolled a total of 314 expectant women. Their characteristics are outlined in Figure 4.1. The ages of the participating women ranged from 18 to 38 years with the median (interquartile range) age was 24 (20 – 27) years.

Figure 4.1: Age distribution
4.1.2 Parity categories

Majority of the participants were multiparous (252, 80.3%) while the rest were primiparous women (62, 19.7%). Parity among the multiparous women ranged from one to seven children. Those who had four children or more constituted 11.8% of the respondents (Figure 4.2).

![Bar graph showing parity categories](image)

**Figure 4.2: Parity categories.** Parity Zero indicates a woman has never given birth, One has given birth once, two has given birth twice, three has given birth thrice and ≥4 has given birth four or more times.
4.2 Prevalence of malaria based on Microscopy, rapid ICT test and Microscopy and PCR

Table 4.1 presents the results on the malaria prevalence among the sampled women based on the three diagnostic techniques that were utilized in the study. Overall, 62 (19.7%) and 44 (14.0%) women diagnosed as positive for malaria by rapid ICT test and microscopy respectively. Further, 20 of the 38 samples (52.6%) subjected to the confirmatory test (PCR) were positive for malaria. Those who were found positive for malaria by both microscopy and PCR were 64 (20.4%).

Table 4.1 Results on malaria prevalence based on Microscopy, rapid ICT and Microscopy and PCR

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid ICT</td>
<td>62</td>
<td>19.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>44</td>
<td>14.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy and PCR</td>
<td>64</td>
<td>20.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>252</td>
<td>80.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>86.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>79.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3 Evaluation of the performance of rapid ICT and microscopy

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed for the rapid ICT. In the present study, malaria cases diagnosed using PCR and microscopy were regarded as the "true positives", else "true negatives". The sensitivity and specificity of rapid ICT was 84.4% (95% confidence interval (CI)
73.6% - 91.3%) and 96.8% (95% CI 93.8% - 98.4%) respectively while that of microscopy was 68.8% (95% CI 56.6% - 78.8%) and 100.0% (95% CI 98.5% - 100.0%) respectively. The overall diagnostic accuracy of rapid ICT was 94.3% (95% CI 91.1% - 96.3%) while that of microscopy was 93.6% (95% CI 90.4% - 95.8%) as shown in Table 4.2.

Table 4.2: Assessment of validity of ICT and microscopy

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=64) %</td>
<td>(n=250) %</td>
<td>No. %</td>
<td>No. %</td>
<td>%</td>
</tr>
<tr>
<td>Rapid ICT</td>
<td>54</td>
<td>84.4</td>
<td>242</td>
<td>96.8</td>
<td>52 of 62</td>
</tr>
<tr>
<td>Microscopy</td>
<td>44</td>
<td>68.8</td>
<td>250</td>
<td>100</td>
<td>44 of 44</td>
</tr>
</tbody>
</table>

†CI = Confidence interval

4.4 Assessment of the agreement between rapid ICT, microscopy and microscopy and PCR

Cohen's $\kappa$ was run to determine if there was agreement between rapid ICT, microscopy and microscopy and PCR in the diagnosis of malaria. The findings are presented in Table 4.3. There was moderate level of agreement observed when microscopy was assessed against ICT ($\kappa = 0.517; p <0.001$). Additionally, high level of agreement was observed between the results obtained from ICT and those obtained by a combination of microscopy and PCR ($\kappa = 0.821; p <0.001$).
Table 4.3 Assessment of the agreement between rapid ICT, microscopy and microscopy and PCR

<table>
<thead>
<tr>
<th>Test</th>
<th>Total (n=314)</th>
<th>Positive (n=62)</th>
<th>Negative (n=252)</th>
<th>(\kappa)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microscopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
<td>34 (77.3)</td>
<td>10 (22.7)</td>
<td>0.517</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>270</td>
<td>28 (10.4)</td>
<td>242 (89.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Microscopy and PCR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>64</td>
<td>54 (84.4)</td>
<td>10 (15.6)</td>
<td>0.821</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>250</td>
<td>8 (3.2)</td>
<td>242 (96.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5 Assessment of the effect of age and parity on malaria prevalence

Table 4.4 shows the findings based on the evaluation of the effect of age and parity on malaria prevalence. Overall there was a decrease in malaria prevalence with decreasing age of women. However, this trend was not significant. The highest prevalence of malaria was observed among primiparous women (32.3%) with lower prevalence of malaria being reported among women of higher parity. Being primiparous was associated with 4.8 times increment in the risk of being diagnosed with malaria when compared to the women whose parity was three or more (Odds ratio (OR) 4.830 (95% CI 1.884-12.383); p=0.001). Further, women whose parity was one had a four-fold increase in likelihood of having malaria as compared to their counterparts whose parity was three and above (OR 4.090 (95% CI 1.655-10.107); p=0.001). Primiparous women were more likely to be diagnosed with malaria when compared to multiparous women (OR 2.251 (95% CI 1.206-4.201); p=0.010).
Table 4.4: Evaluation of the effect of age and parity on malaria

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>OR* (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤19</td>
<td>60</td>
<td>15</td>
<td>25.0</td>
<td>45</td>
<td>75.0</td>
<td>1.739 (0.834- 3.628)</td>
<td>0.137</td>
</tr>
<tr>
<td>20 – 24</td>
<td>111</td>
<td>26</td>
<td>23.4</td>
<td>85</td>
<td>76.6</td>
<td>1.596 (0.853-2.985)</td>
<td>0.141</td>
</tr>
<tr>
<td>≥25</td>
<td>143</td>
<td>23</td>
<td>16.1</td>
<td>120</td>
<td>83.9</td>
<td>Reference (1.000)</td>
<td></td>
</tr>
<tr>
<td>Parity categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>62</td>
<td>20</td>
<td>32.3</td>
<td>42</td>
<td>67.7</td>
<td>4.830(1.884-12.383)</td>
<td>0.001</td>
</tr>
<tr>
<td>One</td>
<td>87</td>
<td>25</td>
<td>28.7</td>
<td>62</td>
<td>71.3</td>
<td>4.090(1.655-10.107)</td>
<td>0.001</td>
</tr>
<tr>
<td>Two</td>
<td>87</td>
<td>12</td>
<td>13.8</td>
<td>75</td>
<td>86.2</td>
<td>1.623(0.605-4.355)</td>
<td>0.333</td>
</tr>
<tr>
<td>≥3</td>
<td>78</td>
<td>7</td>
<td>9.0</td>
<td>71</td>
<td>91.0</td>
<td>Reference (1.000)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>62</td>
<td>20</td>
<td>32.3</td>
<td>42</td>
<td>67.7</td>
<td>2.251(1.206-4.201)</td>
<td>0.010</td>
</tr>
<tr>
<td>Multiparous</td>
<td>252</td>
<td>44</td>
<td>17.5</td>
<td>208</td>
<td>82.5</td>
<td>Reference (1.000)</td>
<td></td>
</tr>
</tbody>
</table>

*Odds ratio
Binary logistic regression revealed that a year increase in age was associated with about 3% decrease in probability of suffering from malaria (adjusted odds ratio (aOR) 0.972 (95% CI 0.952-0.992); p=0.007). On the other hand, a unit change in parity was found to be associated with 36% reduction of the risk of being diagnosed with malaria (aOR 0.643 (95% CI 0.484-0.854); p=0.002) (Table 4.5).

Table 4.5: Odds Ratio tests of the effect of age and parity on malaria

<table>
<thead>
<tr>
<th>Variable</th>
<th>aOR* (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.972(0.952-0.992)</td>
<td>0.007</td>
</tr>
<tr>
<td>Parity</td>
<td>0.643(0.484-0.854)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Adjusted odds ratio

4.6 Evaluation of the effect of age and parity on malaria parasitaemia level

Malaria parasitaemia levels were assessed amongst the 44 expectant women who were reported as positive for malaria using microscopy. The parasitaemia levels in this group ranged from 40 to 73000 parasites/µl of blood. The geometric mean parasite density was 2026 (95% CI 1196 - 3434) parasites/µl of blood. Fourteen women (31.8%) had low parasite density (<1000 parasites/µl of blood) while 17 and 13 women (38.6%; 29.5%) had medium (1000-4999 parasites/µl of blood) and high parasite densities (≥5000 parasites/µl of blood) respectively as shown in Figure 4.3 below. There was no significant difference in the parasite densities in the infected multiparous and primiparous women (p=0.748). Analysis of variance of parasite densities by age groups revealed no
statistically significant variations (p=478). A weak negative correlation between age and parasite levels was observed though not significant (r=-0.117; p=450). Similarly, the correlation between parity and parasite levels was not statistically significant (r=0.015; p=9.25).

**Figure 4.3**: Parasitaemia in women who were positive for malaria by microscopy. Data was presented as parasite density (parasite / µl of blood): Low had 40-999, medium had 1000-4999 while high had greater than or equal to 5000 parasites per microlitre of blood.
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Evaluation of the performance of rapid ICT and microscopy in detection of malaria parasites in pregnant women

Although microscopy is the gold standard method currently acceptable in diagnosis of malaria in hospital, the use of rapid ICTs is being promoted as a part of the efforts to control malaria among the pregnant women. The data generated in the present study from rapid ICT and microscopy indicated that the higher number of positive cases recorded by rapid ICT compared to microscopy is attributed to the fact that it has higher sensitivity. According to Goldring (2004), Desai et al. (2007) and Cox-Singh et al. (2008), the higher sensitivity of rapid ICT compared to microscopy can be expected because during pregnancy, malaria parasites disappear from peripheral circulation and remain undetectable through microscopic examination due to sequestration of the parasites in the placenta. In contrast, parasite antigen is present in circulation and can be readily identified by rapid ICT (Goldring, 2004).

In the present study, rapid ICT recorded a higher sensitivity and accuracy compared to microscopy thus revealing its reliability as a complementary tool in malaria diagnosis among expectant women in areas with low parasite density levels. According to Harchut et al. (2013) the ability of rapid ICT to detect malaria parasites below the threshold of microscopy supports its reliability in the detection of malaria in pregnancy. High level of agreement observed between the results obtained from rapid ICT and those obtained by a
combination of microscopy & PCR shows that rapid ICT it can be used to complement microscopy in parasitological diagnosis of malaria among expectant women.

The low prevalence of malaria parasite recorded by microscopic examination in the present study may be an indication of false negative cases. This may be attributed to lack of technical expertise and unfamiliarity of laboratory personnel to malaria parasite. The presence of false negatives in microscopy diagnosis predisposes to pregnant women suffering from malaria not receiving the drugs thus leading to poor maternal and foetal outcomes. The false positives from microscopic tests in the present study highlight the urgent need for additional training of laboratory personnel on microscopic diagnosis of malaria. This is particularly important in the tropics where the presence of other blood parasites and pathogens may interfere with accurate detection of malaria parasites (Kyabayinze et al., 2010). The inability to perform effective diagnosis of malaria greatly increases indiscriminate use of antimalarials among pregnant women hence increasing rate of drug resistance.

Although pregnant women who were under antimalarial treatment 2 weeks before initiating the project and during the course of this study were excluded from the present study, false positives were recorded by rapid ICT using microscopy and PCR as a confirmatory test. However, this may have resulted because antigens can remain detectable in the blood stream several weeks after recovery from malaria (Hviid, 2010; Lee et al., 2010; Kyabayinze et al., 2010). The performance of malaria rapid diagnostic kits is also affected by antigenic variation in *Plasmodium falciparum* where deletion of
HRP2 gene is associated with false negatives results (Maltha et al., 2013). This study did not determine the P. falciparum antigenic types present in the study population and thus the role of antigenic variation in detection of false negatives is not clear and should be the focus of future studies. Other causes of false positive and false negative may be due to procedural aspects when conducting the tests and inappropriate storage temperatures. However, in the present study, the efficiency of rapid ICT was validated by formation of control band in all the tests. Therefore, there is need to investigate the factors that may lower the efficiency of this promising rapid technique in malaria diagnosis.

5.1.2 Malaria prevalence in relation to parity and age

Malaria infection caused by Plasmodium falciparum has been associated with parity and age as major predictors of disease prevalence during pregnancy (Johnson et al., 2004; Drakeley et al., 2006). In the present study, age and parity were not significant predictors of malaria prevalence among the expectant women attending antenatal clinic. This may be due to the fact that all pregnant women have diminished cell mediated immunity hence are easily prone to malaria infection (Desai et al., 2007). This finding is consistent with findings of a study conducted in Sudan in an area of unstable malaria transmission which showed that pregnant women are highly susceptible to malaria infection, and malaria prevalence is high regardless of their ages and parity (Ali et al., 2011). An earlier study conducted by Johnson et al. (2004) among expectant women had reported that there was no association between age and parity on malaria prevalence among the pregnant women. The study attributed these findings to the fact that pregnant women in non-endemic areas and those of unstable malaria transmission are susceptible
to malaria infection because they have not developed adequate immunity against malaria from previous exposure. Findings from a study by Desai et al. (2007) also attributed the vulnerability of pregnant women to malaria infection to depletion of cell-mediated immunity which is responsible for fighting malaria infection. However, findings in the present study differ from previous studies conducted in Luanda (Angola), Nigeria, Mali and South East Asia which reported that large number of pregnant women affected by malaria are multiparous (Dicko et al., 2003; Campos et al., 2012; Oliver et al., 2014). In addition, according to Rogerson et al. (2007), parity is a predictor of malaria prevalence during pregnancy and primiparous women are more susceptible to malaria. This raises the need for more research to validate the findings.

5.1.3 Malaria parasitaemia in relation to parity and age

In the present study parity and age were not significant predictors of malaria parasitaemia. This may be due to the fact that the study population was in a non-endemic area for malaria hence the parasite density level of pregnant women was comparable due to lack of acquired immunity caused by continuous exposure to the parasite. Furthermore, according to Rogerson et al. (2007), in malaria non endemic areas the placenta of pregnant women is immunologically naïve due to lack of continuous exposure to malaria parasite and thus harbor sequestration of parasites in large numbers. Findings from the present study indicating that parasitaemia levels were high irrespective of parity or age confirms earlier findings by Desai et al. (2007) and Ali et al. (2011) who reported high parasite levels in women of different parity status and ages. In contrast, the finding from the present study differs from a study conducted by Arabidor et al. (2007) among the
expectant women which found out that the levels of malaria parasite density decreases with increase in the number of pregnancies. Therefore, more studies are necessary to validate the findings.

5.1.4 Effect of age and parity on malaria prevalence and parasitaemia level

In the present study, Odds ratio tests showed that age and parity are not significant predictors of malaria prevalence and parasitemia. However, it was interesting to note that an increase in age was associated with a decrease in probability of suffering from malaria infection. In addition a unit change in parity was found to be associated with a reduction of the risk of being diagnosed with malaria. Microscopic examination and rapid ICT test recorded higher prevalence among primiparous women compared to multiparous women. This may be due to physiological stress experienced during pregnancy and high parasite sequestration in the intervillous space of placenta (Kyabayinze et al., 2010). In contrast to findings in the present study, a study by Oliver et al. (2014) among the expectant women reported high number of multiparous women affected as compared to primiparous women. In addition, according to Cohee et al. (2014) reliability of microscopy in malaria diagnosis in pregnant women is lower at low parity levels compared to high parity levels due to low density of parasites in peripheral circulation. Therefore, other studies would be helpful to confirm these findings for effective malaria control among the expectant women.
5.2 Conclusions

i) From this study, it can be concluded that, rapid ICT recorded a higher sensitivity and accuracy in detection of *Plasmodium falciparum* hence proving to be a suitable and reliable alternative in diagnosis of malaria among expectant women in low malaria parasite density areas.

ii) Age and parity are not significant predictors of malaria prevalence and parasitaemia among expectant women.


### 5.3 Recommendations

i) This study recommends the adoption of rapid ICT as a complementary tool in diagnosis of malaria among expectant women in areas with limited resources, where microscopy is difficult to implement and in low malaria density areas.

ii) Effort should be geared towards control of malaria among all expectant women irrespective of age and parity.
REFERENCES


\textbf{World Health Organization (2013).} List of rapid diagnostic test (RDTs) kits for malaria (Accessed August 28, 2014). Available at: http://www.wpro.who.int/sites/rdt

APPENDICES

APPENDIX I: A map of Kericho County showing the location of study sites
##APPENDIX II: Reaction mix for each tube of Outer PCR of k-13 propeller gene

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Final conc /reaction volume</th>
<th>Volume (µL) per 30 µLtube</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCR buffer</td>
<td>1 X</td>
<td>3 µL</td>
</tr>
<tr>
<td>25mM MgCl2</td>
<td>1.5mM</td>
<td>3.6 µL</td>
</tr>
<tr>
<td>20mM dNTPs</td>
<td>0.2mM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>10 µM primer (K13-1)</td>
<td>100nM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>10 µM primer (K13-4)</td>
<td>100nM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>Taq DNA polymerase (5 u/ µL)</td>
<td>1u/PCR</td>
<td>1.2 µL</td>
</tr>
<tr>
<td>Double distilled water</td>
<td></td>
<td>20.3 µL</td>
</tr>
<tr>
<td>DNA template</td>
<td></td>
<td>1 µL</td>
</tr>
</tbody>
</table>
## APPENDIX III: Reaction mix for each tube of Nested PCR of k-13 propeller gene

<table>
<thead>
<tr>
<th>Reagents for PCR premix</th>
<th>Final conc /reaction vol</th>
<th>Volume (µL) per 30 µL tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCR buffer</td>
<td>1X</td>
<td>3 µL</td>
</tr>
<tr>
<td>25mM MgCl2</td>
<td>1.5mM</td>
<td>3.6 µL</td>
</tr>
<tr>
<td>20mM dNTPs</td>
<td>0.2mM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>10 µM primer (K13-2 )</td>
<td>100nM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>10 µM primer (K13-3)</td>
<td>100nM</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>Taq DNA polymerase (5 u/ µL)</td>
<td>1u/PCR</td>
<td>1.2 µL</td>
</tr>
<tr>
<td>DNase free water</td>
<td></td>
<td>19.3 µL</td>
</tr>
<tr>
<td>DNA template</td>
<td></td>
<td>2 µL</td>
</tr>
</tbody>
</table>
APPENDIX IV: Questionnaire

Performance of rapid immunochromatographic test and the effects of age and parity on malaria among expectant women in Kericho County

Patient number (OP/IP): 

Age in years: 

Parity (No. of past deliveries and abortions): 

Are you currently on anti-malarial drugs  YES  NO

Have you been on anti-malarial drugs the last two weeks?  YES  NO
APPENDIX V: Research Authorization by Ministry of Medical Services

MINISTRY OF MEDICAL SERVICES

Telegram: "MEDICAL", Kericho
Telephone: Kericho (052) 31191
Email: kerichodistricthospital@yahoo.com
When replying please quote
ER0935/2014

Dear Dr. Mbei & Team,

RE: RESEARCH AUTHORIZATION

Reference is made to your request received on 23rd October 2013 on the above mentioned subject.

We are pleased to inform you that your request to carry out a research entitled "Comparison of Rapid Field Immunochromatographic Test to Expert microscopy for the detection of plasmodium Falciparum in pregnant mothers in our facility" has been approved.

The Hospital Ethics and Research committee will discuss with you on the other issues pertaining to logistics and other requirements before commencement of the actual research study.

Note that, the committee expects maximum cooperation and adherence to ethical issues as you embark on your research study. Feedback on the findings of the study should be given to the Hospital on completion for the purpose of quality care and improvement of the Hospital.

Thanks in advance for your cooperation.

Yours faithfully

DR. K. OKUMU
CHAIRMAN
ETHICS & RESEARCH COMMITTEE
KERICHO DISTRICT HOSPITAL
APPENDIX VI: Research Authorization by Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com  P.O. Box 43844, 00100
dean-graduate@ku.ac.ke  NAIROBI, KENYA
Website: www.ku.ac.ke  Tel. 8710901 Ext. 57530

Our Ref: 156/24975/12  Date: 25th October, 2014

The Principal Secretary,
Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. ANNASTESIA W. NJERU REG. NO. 156/24975/12

I write to introduce Ms. Njeru who is a Postgraduate Student of this University. She is registered for a M.Sc. degree programme in the Department of Zoological Sciences in the School of Pure & Applied Sciences.

Ms. Njeru intends to conduct research for a thesis Proposal entitled “Performance of Rapid Immunochromatographic Test and Microscopy in Determination of Malaria Prevalence among Expectant Women in Kericho County Hospitals, Kenya”.

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

DNN/cao
APPENDIX VII: Approval of Research Proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@kzu.ac.ke
Website: www.kzu.ac.ke

Internal Memo

FROM: Dean, Graduate School
TO: Ms. Annastasia Wangari Njeru
     C/o Zoological Sciences Dept.
     KENYATTA UNIVERSITY

DATE: 25th October, 2014

REF: 156/24975/12

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting of 22nd October, 2014 approved your M.Sc. Research Proposal Entitled “Performance of Rapid Immunochromatographic Test and Microscopy in Determination of Malaria Prevalence among Expecient Women in Kericho County Hospitals, Kenya”.

You may now proceed with your Data collection, subject to clearance with the Principal Secretary, Higher Education, Science and Technology.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University’s Website under Graduate School webpage downloads.

Thank you,

DAVID M. NJOROGE
FOR DEAN, GRADUATE SCHOOL

cc. Chairman, Zoological Sciences Dept.

Supervisors:

1. Dr. Joshua Mutiso
   C/o Zoological Sciences Dept.
   KENYATTA UNIVERSITY

2. Dr. Erick Mbui
   School of Health Sciences
   University of Kabangia
   C/o Zoological Dept.
   KENYATTA UNIVERSITY

DNN/cao
APPENDIX VIII: Formal approval by Institutional Research and Ethics Committee

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 334711/2/3
Reference: IREC/2013/18
Approval Number: 0001043

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4696
ELDORET
27th August, 2013

Dr. Erick Mibe & Team,
Kabianga University College,
P.O. Box 2030-20200,
KERICHO-KENYA.

Dear Dr. Mibe & Team,

RE: FORMAL APPROVAL

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

“Comparison of Rapid Field Immunochromatographic Test to Expert Microscopy for the Detection of Plasmodium Falciparum in Expectant Mothers in Kericho District Hospital, Kenya”.

Your proposal has been granted a Formal Approval Number: FAN: IREC 1043 on 27th August, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th August, 2014. 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.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director- MTRH Dean - SOM Dean - SON
Principal- CHS Dean - SPH Dean - SOD
APPENDIX IX: Informed consent form

Performance of rapid immunochromatographic test and the effects of age and parity on malaria infection among expectant women in Kericho County

Principal Investigator: Annastasia Njeru

Co-investigators: Erick Mibei, Albert Kimutai and Julius Koske

Institutional affiliation

1Department of Zoological Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

2School of Health Sciences, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya

3Department of Biological Sciences, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya

4Department of Clinical Medicine, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya

Description

You are invited to participate in a research study on **Performance of rapid immunochromatographic test and the effects of age and parity on malaria among expectant women in Kericho County.**

Malaria is a parasitic infection transmitted by the female anopheline mosquito and current diagnosis is through the conventional peripheral blood microscopy. This method has limitations; for example, its sensitivity decreases as the density of malarial parasites in the blood decreases and is unable to detect all infections as parasites can be sequestered...
in the placenta. Such sub-microscopic infections increase the risk of anaemia and poor foetal outcomes in seemingly aparasitaemic pregnant women. Emerging evidence suggests that Rapid field ICTs are capable of detecting placental malaria better than microscopy and may detect these sub-microscopic infections. These rapid tests are now available and diagnose malaria within 10 minutes unlike microscopy which takes at least 30 minutes. The main purpose of this study is to compare the performance of the rapid field immunochromatographic strip tests to microscopy with a view of recommending the adoption of RDTs for detection of malaria in pregnant women and increase the detection of Malaria cases in pregnant women and hence reduce risk of maternal and perinatal mortality.

**Study procedures**

If you agree to participate in the study, you will be asked to provide five milliliters of venous blood which will be drawn into an EDTA-coated syringe for thick and thin blood film preparation, rapid test, and PCR. Your blood sample will be frozen and will be stored with a number assigned linked to your name instead of your name. Your name will also not appear on the questionnaire and all the samples will be destroyed at the end of the study.

If you are diagnosed with malaria infection using the standard laboratory tests, you will be treated with antimalarial drugs. You will not be treated according to the rapid test results as we are not yet sure if it is accurate.
Voluntary participation

Your decision not to participate in this study will not affect the care you will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time (verbally) without affecting the care that you will receive. During the interview, you can choose not to answer any particular question.

Discomfort and risks

You may feel a slight discomfort resulting from the blood draw.

Benefits

There will be no immediate benefits from your participation in the study. When the study results are known and if the rapid tests are acceptable in terms of accuracy, everyone who comes to the clinic could benefit from having this test available to diagnose malaria and receive the right treatment within a very short time.

Compensation

There will be no monetary compensation for participation in this study.

Confidentiality statement

The records concerning your participation are to be used only for the purpose of this research project. Your name will not be used on any study forms or labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study...
will be kept strictly confidential. Only members of the study team will have access to
information linking your name with your study number. These includes,

The Protocol Director, Principal Investigator

The University of Kabianga and Kenyatta university Administrative Panel on Human
Subjects in Medical Research and any other unit of the two Universities as necessary
Research Staff

Questions and freedom to withdraw from the study

You can withdraw from the study at any time without affecting your present or future
medical care at the clinic. If you have any questions, concerns or complaints about this
research study, its procedures, risks and benefits, or alternative courses of treatment, you
should ask,

The Protocol Director, Principal Investigator

The Institutional Review and Ethics Committee (IREC)

Results publication

When the researchers have analyzed the data, the results and the explanation of its
implications will be posted at the clinic for everyone’s information.

Time involvement

Your participation in this experiment will take approximately 15 minutes for the blood
draw (which will be done as part of your prenatal visit).

Participant’s statement

I have been informed verbally and in writing about this study and understand what is
involved. I also know whom to contact if I need more information. I understand that
confidentiality will be preserved. I also understand that I am free to withdraw from the study at any time without affecting the care I normally receive at the clinic. I agree to participate in this study as a volunteer subject.

______________________________
Date Name of participant

______________________________
Signature (or thumb print or cross) of participant

______________________________
Date Name of witness

Signature of witness

**Investigator’s statement**

I, the undersigned, have defined and explained to the volunteer in a language she understands, the procedures of this study, its aims and the risks and benefits associated with her participation. I have informed the volunteer that confidentiality will be preserved and that she is free to withdraw from the trial at any time without affecting the care she will receive at the clinic. Following my definitions and explanations the volunteer agrees to participate in this study.

______________________________
Date Name of investigator who gave the information about the study

Signature: _______________