The prevalence and importance of malaria infections during pregnancy not detected by microscopy or rapid diagnostic testing

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ABSTRACT

With the advent of rapid diagnostic tests (RDT) for malaria detection, parasitological confirmation of malaria diagnoses has become more readily accessible and increasingly more affordable. However, questions are being asked about how useful they are in screening programmes, particularly for pregnant women who very often may harbour placental malaria infections which may not be detected by peripheral blood smear microscopy. It is also necessary to examine how many malaria infections go undetected by RDTs and whether such undetected infections have any negative consequences for women who carry such RDT-negative infections.

This sub-study was conducted as part of a large multi-country trial of intermittent preventive treatment with sulfadoxine pyrimethamine versus intermittent screening and treatment of malaria in pregnancy which in Burkina Faso, Gambia, Ghana and Mali. The study enrolled 5,354 primi- and secundigravidae who attended antenatal clinics at study sites in the four countries from May 2010 to October 2011.

The sensitivity of the RDT to detect peripheral malaria in pregnancy using PCR as the reference declined from 89.3% (95% CI 85.5-92.4) on the day of enrollment to 57.7% (95% CI 46.5-73.0) at delivery. However, the sensitivity of the RDT to detect placental malaria was lower when placental histology was used as the reference ranging from 72% (95% CI 63.3-79.7) for any woman who tested RDT positive at any scheduled ANC visit, to 35.4% (95% CI 26.6-45.0) at the delivery visit. The prevalence of sub-RDT malaria was highest at delivery (6.3%). There was no significant association between sub-RDT malaria infection and low birth weight. Of the women in a sub-sample who were seen at first ANC visit, 39.0% had malaria infections out of which 1.7% were non-falciparum infections presenting as mono-infections or mixed infections with *P.*

falciparum. Clinical symptoms and signs were not sensitive enough to predict a positive RDT test and therefore the presence of malaria parasites.

Women whose malaria infections get missed by RDTs are not at great risk of developing adverse pregnancy outcomes when compared with women who had no malaria. All pregnant women should be screened with RDT if IST was considered to replace IPTp-SP in areas where SP resistance is too high or transmission intensity is very low.

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LIST OF ABBREVIATIONS

ANC	Antenatal Clinic
AL	Artemether-Lumefantrine
AQ/AS	Amodiaquine/Artesunate
CS	Circumsporozoite
CSA	Chondroitin Sulfate A
DALY	Disability Adjusted Life Years
DNA	Deoxy-ribonucleic Acid
EIR	Entomological Inoculation Rate
FIND	Foundation for Innovative New Diagnostics
HRP-2	Histidine-rich Protein 2
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IST	Intermittent Screening and Treatment
ITN	Insecticide Treated Bednets
IPTp	Intermittent Preventive Treatment (of malaria) in pregnancy
LAMP	Loop-mediated Isothermal Amplification
MiP	Malaria in pregnancy
nPCR	Nested Polymerase Chain reaction
PCR	Polymerase Chain Reaction
PfEMP1	Plasmodium falciparum Erythrocyte Membrane Protein 1
pLDH	Plasmodium Lactate Dehydrogenase
PM	Placental malaria
QT-NASBA	Quantitative Nucleic Acid Sequence-Based Amplification
RDT	Rapid Diagnostic Test
rRNA	Ribosomal Ribonucleic Acid
RTQ-PCR	Real Time Quantitative Polymerase Chain Reaction
SP	Sulfadoxine-Pyrimethamine
WHO	World Health Organisation

CHAPTER ONE

Introduction

1.1 Thesis Organisation

This thesis is organized into eight chapters.

Chapter 1 describes how this thesis is organized and also presents a rationale for the study, followed by the aim and objectives of the study.

Chapter 2 presents the background to the study and a review of relevant current literature.

Chapter 3 presents the study design, a description of the study area, study population and the methods used to research into study questions, the outcomes of interest, sampling and sample size determination, data management and analysis and ethical issues of concern and how they were addressed.

Chapter 4 reports the results of a comparison of the sensitivity and specificity of Rapid Diagnostic Tests and microscopy with PCR in the diagnosis of placental malaria and malaria in pregnancy generally.

Chapter 5 reports the results of a comparison of the prevalence of anaemia, placental malaria and low birth weight among pauci-gravid women with microscopic malaria, sub-microscopic malaria and no malaria, attending antenatal clinics in West Africa

Chapter 6 presents estimates of the prevalence of non-falciparum malaria infections among pregnant women attending ANC in four West African countries and the proportion of non-falciparum malaria infections not detected by rapid diagnostic testing.

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Chapter 7 examines the symptomatology of malaria in pregnancy to determine if a score could be developed to predict the need for RDT screening to detect malaria among pregnant women attending ANC.

Chapter 8 discusses the results presented in the previous chapters in the light of current literature. This chapter also summarises the findings emerging from the study and the conclusions, and ends with recommendations for policy and further research.

1.2 Role of the student investigator in this PhD study

This PhD sub-study was nested within a larger non-inferiority trial of "Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine (SP) versus Intermittent Screening and Treatment of Malaria in Pregnancy" which was conducted in Burkina Faso, the Gambia, Ghana and Mali.

The student investigator coordinated the trial at the Ghana site which was located in the Kassena-Nankana District in the Upper East Region of the country. This involved taking part in the overall design and development of the main trial protocol, submission of the trial protocol for ethical approvals in Ghana (including amendments to trial protocol), coordination and supervision of data collection, coordination of laboratory work and supervision of data management.

Concerning the PhD sub-study, the student traveled to London in January 2011 to work with his Supervisor and other members of his Advisory Committee to develop his PhD protocol. The protocol which was developed was subsequently successfully presented to a panel at an Upgrading Seminar in January 2012. Following the seminar, the student was upgraded from the Master of Philosophy (MPhil) level to the Doctor of Philosophy (PhD) level.

The student subsequently discussed plans for the PhD with the Advisory Committee, which led to the decision for the student to conduct PCR assays on filter paper blood spots collected from study participants at the Medical Research Council (MRC) Unit Laboratories in the Gambia from October to November, 2012. The student took part in all the PCR assay processes with supervision and support from MRC staff. The student had to make a further two-week trip to the Gambia in March 2013 to help complete the reading of PCR assay results.

The student was responsible for the statistical analyses that were conducted for the various objectives of the study, with support from Dr. Mathew Cairns, a member of the Advisory Committee. The write-up of this thesis was the sole responsibility of the student with reviews by the Supervisor and Professor Brian Greenwood, a member of the Advisory Committee.

1.3 Publications that have resulted from this study

Two articles have been written and published from this thesis. These publications are:

i. **Williams, J.E**., Cairns, M., Quaye, S.L., Awine, T., Oduro, A., Tagbor, H., Bojang, K., Magnussen, P., Ter Kuile, F., Wokeu, A., Milligan, P., Chandramohan, D., and Greenwood, B. (2015) The performance of a rapid diagnostic test in detecting malaria infection in pregnant women and the impact of missed infections. *Journal of Clinical Infectious Diseases*, doi: 10.1093/cid/civ1198.

ii. **Williams, J.**, Cairns, M., Bojang, K., Coulibaly, S.O., Kayentao, K., Abubakar, I., Akor, F., Mohammed, K., Bationo, R., Dabira, E., Soulama, A., Djimde, M., Guirou, E., Awine, T., Quaye, S.L., Ordi, J., Doumbo, O., Hodgson, A., Oduro, A., Magnussen, P., ter Kuile, F.O., Woukeu, A., Milligan, P., Tagbor, H., Greenwood, B. and Chandramohan, D. (2016) Non-falciparum malaria infections in pregnant women in West Africa. *Malaria Journal* 15: 53 doi: 10.1186/s12936-016-1092-1

In addition to these articles which have been published, the student plans to develop at least one other manuscript by conducting further analyses on the placental malaria data obtained from this study.

1.4 Rationale

Rapid diagnostic testing for malaria has become increasingly important in the diagnosis of malaria in many endemic countries. This is largely as a result of WHO (World Health Organisation) recommendation that as much as possible the diagnosis of malaria should be based upon parasitological confirmation of the presence of malaria parasite in the bloodstream of a person suspected to be infected, as well as the increasing commercial availability of rapid diagnostic tests (RDTs). Some of the difficulties associated with blood smear microscopy have largely been overcome by the availability of cheaper RDTs with better sensitivity. However, as RDTs have become more accessible and consequently more utilised in many settings where malaria testing was previously inaccessible or unreliable, questions are being asked about how useful they are in screening programmes, particularly for pregnant women who very often may harbour placental malaria infections which may not be detected by peripheral blood smear microscopy. It is also necessary to examine how many malaria infections go undetected by RDTs and whether such undetected infections have any negative consequences for women who carry such RDT-negative infections. While considering the possibility of screening of pregnant women and treatment of those infected instead of IPTp (Intermittent Preventive Treatment in pregnancy), it is necessary to determine whether these RDT-negative malaria infections are of any significant consequence for the health of the woman and her baby.

Even though non-falciparum infections are estimated to be of low prevalence in West African populations in general and among pregnant women in these populations in particular, very little is known about the true burden of these infections among the affected populations. While many RDTs have been developed to detect both falciparum and non-falciparum malaria, it is necessary to determine whether RDTs are able to detect these infections effectively.

As RDT use becomes more universal there is the need to examine ways in which their use can be optimised in settings where supplies cannot always be guaranteed. Furthermore, it is not known how feasible it is to integrate screening of pregnant women using RDTs in busy antenatal clinics in sub-Saharan Africa where midwives are already overstretched with many tasks. Therefore, restricting the number of pregnant women who need screening with RDT at antenatal clinics using a clinical symptoms and signs based algorithm also needs to be examined.

Answers to the following questions are needed to make evidence-based policies on screening and treatment instead of IPTp to control malaria in pregnancy in the West Africa:

- What is the prevalence of sub-microscopic malaria infections in pregnancy?
- Do sub-microscopic malaria infections in pregnancy have clinical consequences?
- What is the prevalence of non-falciparum malaria in pregnancy as revealed by PCR testing?
- Could a symptoms and signs based criterion be useful as a screening tool to detect pregnant women who will need further screening using RDTs

1.5 Aim and Objectives

1.5.1 Aims

To address the knowledge gap in the understanding of the epidemiology and consequences of sub-microscopic malaria infections in pregnancy and to contribute to the evidence base under-pinning polices for control of malaria in pregnancy in West Africa.

1.5.2 Objectives

1. To determine the accuracy of RDTs for detecting placental malaria.
2. To determine accuracy of RDTs for detecting sub-microscopic peripheral malaria parasitaemia in pregnancy.

3. To estimate the prevalence and effects of submicroscopic malaria infections among pauci-gravid pregnant women attending antenatal clinics in four West African countries.
4. To determine the prevalence of non-falciparum malaria infections among pregnant women attending antenatal clinics in four West African countries.
5. To assess the usefulness of symptom and signs-based scores to predict malaria in pregnancy which could be used as a tool to select women needing RDT screening at antenatal clinics.

CHAPTER TWO

Literature Review

2.1 Burden of malaria

Malaria remains a major public health problem in many parts of the world. According to the World Health Organisation (WHO), 91 countries, mainly in tropical and some subtropical regions of the world are currently classified as malaria-endemic (World Malaria Report, 2016). The WHO estimates that the number of malaria cases rose from 233 million in 2000 to 244 million in 2005 but has since declined to 212 million in early 2015.The number of deaths due to malaria is also estimated to have declined from 985,000 in 2000 to 429,000 in 2015. Most of these deaths occurred mainly in children aged under 5 years in Africa. Even though these statistics suggest that the burden of malaria is declining, it still remains a major public health issue, particularly in sub-Saharan Africa; its effects are felt mostly among children and pregnant women, who are among the most vulnerable populations in society. It has been estimated that malaria mortality accounts for 42 million disability-adjusted life years (DALYs) globally, representing 3% of the total DALYs worldwide (Breman et al. 2004). In Africa however, malaria mortality is estimated to constitute 10% of total DALYs. In malaria endemic areas, young children suffer most of the burden of malaria-related morbidity and mortality as most individuals acquire significant clinical protection during the first decade of life (Riley et al. 1994).

2.2 Species of malaria

Five species of the malaria parasite are capable of causing disease in humans. These are *Plasmodium falciparum*, which is responsible for the most severe disease and also the most common type of malaria; *P. vivax*, which requires the Duffy blood group antigen to be able to invade red blood cells and therefore is unable to cause disease in many African populations; *P*

malariae, *P. ovale* and finally *P. knowlesi*, which was previously thought to be responsible solely for malaria in macaque monkeys, but is now known to be an important cause of malaria infection in man in South East Asia.

Of the four non-falciparum plasmodium species which cause human disease, *P. vivax* malaria is the commonest, occurring in many parts of the tropical world, from areas in the south Pacific, across Asia and Africa to the western coasts of South America. The parasite has even been found in some temperate regions in Asian countries like China and Korea (Maguire and Baird, 2010). It has long been known, that the inherited absence of the Duffy antigen on the red blood cells in West and Central African populations, where more than 95% of the populations is Duffy negative, spares them from getting vivax malaria. Homozygosity for the Duffy-negative blood group antigen confers almost complete resistance to vivax malaria (Langhi and Bordin, 2006). Lately, questions have been raised about whether Duffy antigen is the sole receptor/mechanism for the invasion of erythrocytes by *P. vivax* parasites. Menard et al. (2010) have shown that, in Madagascar, *P. vivax* has broken through its dependence on the Duffy antigen for invasion of human red blood cells and therefore disease due to this parasite is found in this part of Africa, as well as in Ethiopia and the horn of Africa.

In contrast to *P. vivax*, *P. malariae* and *P. ovale* infections, though not as common as *P. falciparum* are well recognised among West and Central African populations. *P. ovale* malaria is endemic in western Africa and is also found in isolated pockets in South-East Asia and Oceania. *P. malariae* occurs throughout the tropics in isolated foci at relatively low frequencies (Maguire and Baird, 2010). Several studies in West Africa have described the burden of *P. malariae* and *P. ovale* malaria, mainly in children. The prevalence of *P. malariae* has ranged from 4-33% depending upon whether the survey was done in the dry season or wet season and, in some cases, upon the

vegetational zone in which the survey was done. For *P. ovale*, the prevalence ranged between 2.6 and 15%. Greenwood et al. (1987) found the prevalence of *P. malariae* to range between 13% in the wet season to 33% in the dry season among febrile children in The Gambia. The prevalence among afebrile children was 4% in the wet season and 8% during the dry season in this study. In Burkina Faso, Boudin et al. (1991) obtained a prevalence of *P. malariae* ranging between 3.5-25% among children 0-14 years old, with the annual mean being 14.2% for the 0-4year group, 14.0% for the 5-9 year age-group and 10.8% for the 10-14 year age-group.

In the Central Region of Ghana, Afari et al. (1993) obtained a prevalence for *P. malariae* of 20.4% using microscopy among children under five years old. In the Ashanti Region of Ghana, Browne et al. (2000) obtained a prevalence of 10.4% for *P. malariae* in the forest zone compared to 22.8% for the Savanna zone in the same country. They obtained prevalence of 15.5% for *P. ovale* in the forest area compared to 2.6% in the Savanna area. In a longitudinal study of natural malaria transmission in a holoendemic area in Senegal, Trape et al. (1994) found a prevalence of 21.1% for *P. malariae* and 6.0% for *P. ovale* among the population aged 0 - >60 years using microscopy.

2.3 Malaria in pregnancy

Contrary to what pertains in adults who acquire some level of immunity to malaria, pregnant women living in malaria endemic areas are particularly susceptible to malaria, leading to a higher prevalence and greater severity of disease (Brabin 1983). It is estimated that over 50 million women living in malaria endemic areas in the world are exposed to the risk of malaria in pregnancy each year. Malaria in pregnancy is known to cause substantial maternal, fetal and infant morbidity resulting in an estimated 75,000-200,000 infant deaths every year (Steketee et al. 2001; Desai et al, 2007). Studies have shown that malaria in pregnancy (MiP) occurs largely because infected erythrocytes accumulate in the placenta (Walter et al. 1982), with the sequestration of trophozoites

and schizonts, which are usually absent from the peripheral blood, in the placenta (Beeson et al. 2002). Accumulation of parasites in the intervillous space of the placenta is the cause of maternal anaemia as well as low birthweight, prematurity and increased infant mortality (McGregor et al. 1983). Research has shown that the *var2csa* variant of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is the main protein that mediates parasite accumulation in the placenta (Salanti et al. 2003). Placental parasites appear to bind preferentially to chondroitin sulphate A (CSA) (Achur et al. 2000). In endemic areas, MiP is found mainly among primigravid women suggesting that protective immunity to MiP is acquired as a function of parity (Brabin, 1983). Women acquire immunity to MiP by producing antibodies to MiP variant surface antigens in a gravidity-dependent manner (Rogerson et al. 2007; Ricke et al. 2000).

2.4 Non-falciparum malaria in pregnancy

It is known that pregnant women can be infected by all the plasmodium species which cause human disease except *P. knowlesi*, for which data on pregnant women is unavailable. Nevertheless, given the known behaviour of this parasite, infections in pregnant women would be expected to occur. However, studies of MiP are mainly focused on susceptibility and clinical consequences of *P. falciparum* and *P. vivax*. The susceptibility to *P. falciparum* is increased in pregnancy (Diagne et al. 1997). Studies have also shown that pregnant women are at an increased risk of *P. vivax* malaria, though this risk is less pronounced than with *P. falciparum* (Desai et al, 2007) and that vivax malaria during pregnancy adversely affects the outcome of pregnancy. Nosten et al. (1999), studying *P. vivax* malaria among Karen women living in camps on the western border of Thailand, showed that vivax malaria in pregnancy is associated with maternal anaemia and low birthweight, even though these effects were less striking compared to those caused by *P. falciparum* malaria. They also found that *P. vivax* relapses were more common among pregnant women than in non-

pregnant women. In contrast to *P. falciparum*, which is known to cause most of its effects in pregnant women through sequestration by cyto-adhesion to the syncytiotrophoblast layer, there appears to be little evidence that *P. vivax* also produces its effects in pregnant women through this mechanism (Umbers et al. 2011). However, Carvalho et al. (2010) have shown *P. vivax* adhesion to placental cryo-section *in-vitro*.

There is very little literature on the epidemiology of *P. malariae* and *P. ovale* in any population and in pregnant women in particular. Older studies which used microscopy alone tended to report low prevalences of both these species. There is increasing evidence from studies using PCR that the prevalence of these infections may have been underestimated. Studies have also shown that in sub-Saharan Africa, *P. malariae* and *P. ovale* are often present as co-infections with *P. falciparum* (Desai et al. 2007). Studies in Ghana (Mockenhaupt et al. 2000) and Cameroon (Walker-Abbey et al. 2005) found largely mixed infections of *P. malariae* and/or *P. ovale* with *P. falciparum* in pregnant women by PCR. There are no studies of the impact of mono infections of *P. malariae* or *P. ovale* on the outcome of pregnancy.

2.5 Submicroscopic malaria infections in pregnancy

While microscopy for the detection of malaria parasites in peripheral blood has remained the gold standard for malaria diagnosis for a long time, recent advances in diagnostics, particularly PCR methods, have allowed the identification of malaria parasitaemia below the threshold of detection by microscopy. Studies in sub-Saharan Africa have demonstrated high levels of submicroscopic plasmodial infections by PCR in children and non-pregnant adults (Bottius et al. 1996; Wagner et al. 1998). Several studies in sub-Saharan Africa have also shown that submicroscopic plasmodial infections are very common among pregnant women. These low density infections could have resulted from the gravidity or even previous antimalarial treatment the woman had taken

(Kyabayinze et al. 2016). A study in Ghana, conducted in 1998 among 530 pregnant women living in the forest zone of the country found that while 32% of the women had malaria parasitaemia by microscopy, this increased to 63% using PCR (Mockenhaupt et al. 2000) (Table 2.1). This study also found that women who had antimalarials in their blood stream were 1.8 times (*95% CI 1.2-3.0, p value=0.008*) more likely to have submicroscopic infections compared to women who had no antimalarials present in their bloodstream. Similarly, they also found that gravidity independently predicted submicroscopic *P. falciparum* infection in pregnancy in this region. Women in their 3rd or 4th pregnancy were 4.6 (*95% CI 2.4-8.5, p value=<0.0001*) times more likely to have submicroscopic infections compared to primigravid women, while women who had been pregnant five or more times were 6.3 (*95% CI 3.1-12.6, p value=<0.0001*) times more likely than primigravid women to have submicroscopic malaria infections. These authors suggest that submicroscopic malaria infections in pregnant women may lead to mild anaemia and inflammation in seemingly aparasitaemic pregnant women.

In a study in Cameroon among 78 pregnant women who had normal singleton vaginal delivery (Walker-Abbey et al. 2005) found that the prevalence of *P. falciparum* parasitaemia in the peripheral blood by microscopy was 27.5% while PCR gave a prevalence of 82.4%. This suggests a high prevalence of submicroscopic infections in this population. A similar pattern was obtained by Schleiermacher et al. (2001) in Senegal, who found that 29% of blood samples from pregnant Senegalese women were slide positive while 85% were positive by PCR. These studies and others in sub-Saharan Africa (Saute et al. 2002; Mayengue et al. 2004; Mayor et al. 2009; Rantala et al. 2010) have demonstrated the phenomenon of submicroscopic infections where parasite DNA is found in the blood of pregnant women who are blood smear negative.

Table 1 Prevalence of submicroscopic malaria infections in pregnancy

Country	Microscopy (%)	PCR (%)	Submicroscopic (%)	Reference
Ghana	32	63	31	Mockenhaupt et al. 2000
Cameroon	27.5	82.4	54.9	Walker-Abbey et al. 2005
Senegal	29	85	56	Schleiermacher et al. 2001

2.6 Diagnosis of malaria in pregnancy

The diagnosis of malaria remains a challenge particularly in resource poor settings. The severe consequences of malaria among children and pregnant women makes early and accurate diagnosis extremely important. The WHO has consistently advocated early and accurate diagnosis of malaria and appropriate case management as essential to addressing the burden of malaria in pregnancy as one of the main interventions of the Global Malaria Control Strategy (WHO, 2000; Bell et al, 2006). Two main approaches for the diagnosis of malaria in pregnancy are currently used. These are clinical diagnosis made presumptively using signs and symptoms at presentation and laboratory diagnosis based on the various tests which are currently available.

2.6.1 Clinical diagnosis of malaria in pregnancy

Most health care practitioners in sub-Saharan Africa rely on clinical features to make a diagnosis of malaria in pregnancy. The main clinical feature used in this process is the presence of, or a history of, fever (Uneke 2008). Even though this approach may not necessarily be the best, it is often justified in view of the fact that in endemic areas individuals may be parasitaemic most of the time (WHO, 2000b) and the presence of parasites in non-pregnant individuals is not always indicative of disease even though this may not necessarily be true in pregnant women. Clinical diagnosis was also largely accepted in the past when inexpensive and well-tolerated antimalarials were still effective (Biritwum et al. 2000; Ruebush et al. 1995).

According to WHO, clinical diagnosis of malaria may pose problems for the following reasons (WHO, 2000b):

1. There is no clinical picture of malaria or its complications that is unequivocally diagnostic of the infection as all features can be mimicked by other tropical diseases.
2. The presence of parasitaemia does not prove that malaria is the main or only cause of a patient's illness.

The role of clinical features in diagnosing malaria in pregnancy remains unclear. In areas where malaria transmission is low and unstable, malaria in pregnancy is usually symptomatic and acute and is associated with detectable peripheral parasitaemia (Brabin 1985; Shulman et al. 2001; Adam et al. 2005). It had previously been thought that in areas where transmission is stable and intense, malaria in pregnancy was nearly always asymptomatic in spite of the sequestration of infected erythrocytes in the placenta (Brabin1985; Shulman et al. 2001; Steketee et al. 2001). However, Tagbor et al. (2008) working in an area of intense and stable transmission in Ghana concluded that malaria in pregnancy was sometimes symptomatic. They found that the incidence of a history of fever, headache, vomiting, malaise, dizziness or fatigue was substantially higher in parasitaemic primigravid women compared to aparasitaemic primigravid women. Similarly, in a hospital-based descriptive study conducted in rural Mozambique between 2003 and 2005 aimed at characterizing the clinical presentation of malaria in African pregnant women Bardaji et al. (2008) found that symptoms suggestive of malaria such as headache, arthromyalgias and history of fever were very frequent among pregnant women. However, less than a third of them were parasitaemic. In view of the fact that the authors only used peripheral blood smears for malaria detection and the fact that placental malaria is not reflected adequately by peripheral parasitaemia, the prevalence of malaria among those presenting with symptoms was probably higher than they recorded.

2.6.2 Parasite-based diagnosis of malaria in pregnancy

Laboratory diagnosis of malaria involves the use of tests and assays to detect the presence of malaria parasites or antigens in an infected individual. The three main types of tests used are blood smear microscopy, rapid diagnostic tests based on lateral flow immunochromatographic technology and the polymerase chain reaction for the detection of malaria parasite DNA.

2.6.2.1 Blood smear microscopy

The standard laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with either Giemsa, Field's or Wright's stains (Warhurst and Williams, 1996). This has subsequently become the gold standard of malaria diagnosis (Wongsrichanalai et al. 2007). Two types of films can be prepared - the thick and the thin film. The thick film concentrates layers of red blood cells on a small surface and enhances the sensitivity of the blood film technique, while the thin film facilitates species identification, providing greater specificity than the thick film (Moody, 2002). In addition, smear microscopy is used for estimation of parasite density (Murray et al, 2008). It is, therefore, used as a tool to monitor the response of individuals to therapeutic agents. However, microscopy requires well-trained microscopists, good quality laboratory supplies, rigorous maintenance of equipment and well-functioning quality control and quality assurance systems (Wongsrichanalai 2007). It is labour-intensive and time-consuming and its interpretation requires considerable expertise, especially when parasite density is low (Warhurst and Williams, 1996). In pregnant women, *P. falciparum* malaria is associated with sequestration of the parasites in the placenta, thereby reducing the sensitivity of microscopic diagnosis, as the parasites often cannot be found in a peripheral blood film.

2.6.2.2 Rapid diagnostic tests (RDTs)

Since the early 1990s, rapid diagnostic tests for the diagnosis of malaria have become available. The two main groups of RDT in use detect parasite histidine-rich protein 2 (HRP-2) or plasmodium

lactate dehydrogenase enzyme (pLDH). Some RDTs target the parasite glycolytic pathway enzyme aldolase, which is found in all plasmodium species.

HRP 2 is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum* only, expressed on the erythrocyte cell membrane and shown to remain in the blood for up to 28 days after initiation of antimalarial therapy (Karbwang et al. 1996; Richter et al. 2004; Swarthout et al. 2007). Some parasites have a gene deletion for the production of HRP 2. Such mutants may give false negative test (Baker et al, 2005; Lee et al, 2006). Also, a positive rheumatoid factor has been associated with false positive results for HRP 2 (Laferi et al. 1997; Iqbal et al. 2000). However, these reactions are reported to be less frequent when monoclonal IgM antibodies are used instead of IgG antibodies to prepare the test (Grobusch et al. 1999; Mishra et al. 1999).

Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by asexual and sexual stages of the live parasites and it is released from parasite infected red blood cells. It has been found in all five main human malaria species. The blood levels of pLDH and aldolase decline rapidly to undetectable levels after initiation of effective treatment, even though young plasmodial gametocytes produce pLDH and, therefore, a test based on this enzyme may remain positive despite clearance of the asexual forms of the parasite (Miller et al. 2001; Mueller et al. 2007).

Rapid diagnostic tests detect malaria antigen in small amounts of blood, usually 5-15 microlitres, by immunochromatographic assays in which monoclonal or polyclonal antibodies are impregnated in a nitrocellulose membrane strip. The clinical sample moves across the membrane by capillary action (Bell et al. 2006; Wongsrichanalai et al. 2007; Murray et al. 2008). The result is usually a coloured line which is obtained within 5-20 minutes. RDTs are simple to perform, require no electricity, can be taught to lower-level village health workers and are easy to interpret. They have

been produced in different formats such as dipsticks, card tests and plastic cassettes to promote ease of use. The prices of RDTs have been declining since they first appeared on the market. The market price for RDTs in the mid-2000s in developing countries was between US $0.55- US$1.50 per test, depending on the type of test and the quantities ordered, compared to microscopy which cost US$0.12-US$0.40 per smear (Wongsrichanalai et al. 2007). RDT prices have since declined further with UNICEF, an organization which procures large quantities of RDTs annually, documenting a decline in prices from $0.60 per test in 2011 to $0.44 per test in 2015 (UNICEF, 2016).

The WHO estimates that to be a useful diagnostic, RDTs must achieve a sensitivity of greater than 95% (WHO, 2000b). HRP-2 RDTs have given sensitivities of more than 90% in evaluations involving clinical cases (Kilian et al. 1999; Guthmann et al. 2002). However, when HRP-2 was combined with an aldolase assay the non-falciparum sensitivity was reported to be lower (Tjitra et al. 1999; Cho-Min-Naing et al. 2002). Sensitivity of pLDH RDTs has varied among studies. Their sensitivity for *P. falciparum* appears very good (>95%) in some studies whereas it is much lower (80%) in other studies (Gasser, 2000; Singh et al, 2003).

Since RDTs became available, many brands have been produced and are being marketed. Consequently, the level of their use has also increased markedly in recent years.

Initially, good quality performance data were unavailable to enable national malaria control programmes to make decisions on procurement and mass deployment. To address this deficiency, WHO and FIND (Foundation for Innovative New Diagnostics) launched a programme in 2006 to evaluate the performance of commercially available RDTs. A total of 247 products have since been tested in six rounds against prepared blood panels containing patient-derived *P. falciparum* and *P. vivax* parasites. Importantly, assessment has also been made of their thermal stability, as

these tests will be used mostly in countries with warm climate. The sixth round of testing was completed in 2015.

The results from round 6 show consistency in the panel detection scores, positivity rate, false-positive rates and heat stability in reference to those reported in the earlier rounds. The proportion of tests that achieved a panel detection score of ≥75% at low parasite density (200 parasites/microliter) was higher than all the previous rounds for both *P. falciparum* and *P. vivax*. Several RDTs in the six rounds of testing consistently detected malaria at low parasite density had low false positive rates, were stable at tropical temperatures, were easy to use and could detect *P. falciparum* or *P. vivax* infections or both. Also, *P. falciparum* tests targeting HRP2 had higher panel detection scores than those targeting pLDH (WHO 2015).

Some studies have tried to evaluate the use of RDTs among pregnant women. Tagbor et al. (2008) obtained a sensitivity of 97% and a specificity of 85% for the OptiMAL® RDT, a pLDH-type RDT, using peripheral blood microscopy as the standard among a group of pregnant women attending antenatal clinics who were participating in a clinical trial of anti-malarial drugs in pregnancy in Ghana. However, the assay was not compared to the prevalence of placental malaria. A study in Burkina Faso by Singer et al. (2004) found that the prevalence of placental parasitaemia in their study sample was 23% by placental blood microscopy, 51% by PCR and 43% by RDT (MAKROmed®). Using PCR as the gold standard they found that this particular RDT had a sensitivity of 89% and a specificity of 76% for placental malaria.

Kyabayinze et al. (2016) also conducted a prospective study of 990 HIV-uninfected pregnant women attending antenatal clinics in Uganda and Burkina Faso, who were enrolled in the second or third trimester. They compared results obtained from testing blood sampled obtained from the study participants using expert microscopy, two combination types of RDTs (CareStart™ Malaria

pLDH [Pf/PAN] test and CareStart™ Malaria HRP2/pLDH [Pf/PAN] COMBO test) and nested PCR. In comparison with PCR, the HRP2-based RDT performed best, leading the investigators to suggest that HRP2-based tests are the most appropriate RDTs currently available for use during pregnancy even though they missed some PCR-positive tests.

2.6.2.3 PCR and other methods

PCR methods have been developed for the detection of malaria DNA from whole blood. The diagnostic problems associated with microscopy and RDTs and the need for accurate diagnoses of malaria in pregnancy led to the introduction of PCR (Malhotra et al. 2005; Mockenhaupt et al.2006). The small-subunit 18S rRNA and circumsporozoite (CS) genes have been used in the differentiation of plasmodial species (Moody, 2002). The greatest strength of PCR lies in its high sensitivity, demonstrated by its ability to detect five parasites or less per microlitre of blood (Snounou et al. 1993). PCR methods have consistently shown that at least twice as many pregnant women are infected with malaria compared to microscopy (Mockenhaupt et al. 2000; Adam et al. 2005; Malhotra et al. 2005; Walker-Abbey et al. 2005; Mockenhaupt et al. 2006; Adegnika et al. 2006). A good proportion of pregnant women in malaria-endemic areas suffer submicroscopic malaria infections detectable only by molecular methods.

The two main variants of PCR that have been used for malaria detection are nested PCR (nPCR) and real-time quantitative PCR (RTQ-PCR). RTQ-PCR provides quantitative information about infection burden in an individual and has been shown to be more predictive of clinical and adverse perinatal outcomes of malaria than nPCR and RDT (Malhotra et al. 2005).

PCR assays are confronted by some challenges. They are more labour-intensive, time-consuming and costly compared to microscopy. Also, contamination, which leads to the production of false positive results, is common unless tests are conducted under carefully controlled conditions

(Singer et al. 2004).This has, however, not proven to be a major problem when the test has been done with reasonable care.

Another molecular method which has been developed and evaluated for the detection and quantification of malaria parasites is real-time quantitative nucleic acid sequence-based amplification (QT-NASBA). The real-time QT-NASBA for the detection of *P. falciparum* was developed by Schoone et al. (2000). This assay is based on the detection of the 18S rRNA under isothermal conditions. In comparison with RTQ-PCR, this assay has been shown to be faster to perform as results are available after 4 hours compared to 16 hours for RTQ-PCR (Schneider et al. 2005). The sensitivity and specificity of the two types of assays for detecting malaria parasites are comparable. However, QT-NASBA like PCR, is also expensive and relatively labour-intensive compared to microscopy and requires a high level of expertise and a well-equipped laboratory.

To address some of the disadvantages of PCR, loop-mediated isothermal amplification (LAMP) assays, which are cheaper, faster and simpler than PCR have been developed for the detection of malaria infections (Poschl et al. 2010). LAMP is a molecular method involving nucleic acid amplification that relies on autocycling strand-displacement DNA synthesis. The main advantage of this method is that no denaturation of the DNA template is required and therefore the assay can be conducted under isothermal conditions (Notomi et al. 2000). Species specific LAMP assays for the four common types of human malaria parasites have been developed (Poon et al. 2006; Han et al. 2007). Initial evaluations of the use of LAMP assays in malaria diagnosis are beginning to appear in the literature. Poschl et al. (2010), working in northern Thailand applied three methods, microscopy, nested PCR and LAMP assay to 105 human blood samples and concluded that LAMP was as reliable as nPCR and more reliable than microscopy in the examined blood samples. Similar results and conclusions had earlier been obtained on a smaller sample size by Han et al. (2007),

also working in northern Thailand. More recent evaluations of malaria LAMP assays have concluded that: LAMP detected a higher number of malaria infections than RDT and was user-friendly (Cook et al. 2015); LAMP had a diagnostic accuracy similar to that of nested PCR with a greatly reduced time to result and was superior to expert microscopy (Polley et al. 2013; Hopkins et al. 2013). LAMP assay use has so far not been evaluated in pregnant women. However, the potential for their use in the detection of malaria in pregnancy under field conditions appears promising.

2.7. Placental malaria

The presence of placental malaria can be detected by microscopy of thick or thin smears of placental blood, impression smears of placental tissue or histological examination of placental biopsies.

2.7.1 Placental blood smears

The diagnosis of placental malaria in many endemic areas, particularly in sub-Saharan Africa, still depends on microscopy of placental blood smears or placental impression smears (Uneke 2008). Placental blood smear microscopy permits the detection of malaria parasites and pigment and is easier, cheaper and faster to perform than histology (Brabin et al. 2004). They also allow the speciation of malaria parasites (Ismail et al. 2000; Galbraith et al. 1980), which is difficult with histological examination. They also avoid the confusion that often results from mix-ups between formalin pigment and malaria pigment in histological slides (Ismail et al. 2000). However, placental blood smears are often difficult to read because of the presence of cellular debris and larger numbers of white blood cells (Singer et al. 2004). Overall, placental microscopy is less sensitive for diagnosing placental malaria compared to placental histology (Okoko et al. 2002; Rogerson et al. 2003).

2.7.2. Placental histology

Several histological changes in placentae infected by malaria parasites have been described. These involve the villi and villous surfaces and are considered a direct consequence of parasite accumulation in the placenta (Walter et al. 1982). These changes include perivillous fibrinoid deposits, excessive syncytial knotting, necrosis of the syncytiotrophoblast, proliferation of the cytotrophoblast, loss of syncytial microvilli and trophoblastic membrane thickening (Walter et al. 1982; Bulmer et al. 1993; Ismail et al. 2000; Galbraith et al. 1980; Yamada et al. 1989).

Placental malaria was first classified by Bulmer et al. in 1993. This classification has since been modified by Ismail et al. (2000) as follows:

1. No infection: No malaria parasites or pigment present in the placenta
2. Acute Infection: Malaria parasites present and pigment deposition minimal or absent
3. Chronic Infection: Malaria parasites are present and pigment deposition is present in fibrin or cells within fibrin in significant quantities
4. Past Infection: Malaria parasites are absent but pigment is present.

Other authors have tried to modify this classification further or even offered new classifications. Rogerson et al. (2003) further divided the category of chronic active infections by the presence or absence of pigment in the cytoplasm of monocytes because of the strong association between pigment in macrophages and low birth weight. Muehlenbachs et al. (2010) have developed a 2-parameter grading scheme for placental malaria, which measures inflammation and pigment deposition, based on studies of the placentas of women from Tanzania and Thailand. However, this scheme is yet to be evaluated in clinical trials to determine its suitability for use compared to the existing classification.

Placenta infection is used widely as a standard indicator of malaria infections in pregnancy in epidemiological studies (Cottrell et al. 2005). In spite of its usefulness in research, placental

histology can only be done when pregnancy has come to an end and when the deleterious effects of malaria on the mother and foetus already have taken place. Also, placental histology is expensive and labour intensive and it is not available in most settings in sub-Saharan Africa (Rogerson et al. 2003). Therefore, alternative tests which can predict the presence of placental malaria during pregnancy are greatly needed.

CHAPTER THREE

Materials and Methods

3.1 Study Design

This study was part of a non-inferiority trial of standard Intermittent Preventive Treatment (IPT) with Sulfadoxine-Pyrimethamine (SP) versus intermittent screening with RDTs and treatment with Coartem® for those who tested positive for malaria among pregnant women in Ghana, Burkina Faso, Mali and the Gambia.

3.2 Main trial sample size

The trial was powered to show that Intermittent Screening and Treatment (IST) is not inferior to SP-IPTp in preventing low birth weight (LBW). Review of the literature indicates that the prevalence of LBW (birth weight <2,500 g) in primigravidae or secundigravidae who received a full course of SP-IPTp is likely to be about 10% with some variation between the four sites. To demonstrate with 90% power and at a 0.025% significance level that the prevalence of low birth weight among infants born to mothers in the IST arm is not more than 3% above that of infants born to mothers in the IPT arm, approximately 2,000 women were needed in each group. This sample size gave the study 90% power at a 0.025% significance level to show that the difference in mean birth weight between study groups was less than 50g, the largest difference between groups that would be acceptable as the basis for a change in policy. A study of this size had similar power to show that the difference in haemoglobin concentration between groups was less than 0.2g/dl. A study of this size also had around 90 % power to show that the difference in the prevalence of placental malaria between groups was less than 5%. However, we estimated that only about 50% of women would deliver in a hospital or health centre and provide a placental

biopsy. With a reduced sample size of 2500, the study had 80% power to show that the difference in placental malaria between groups was less than 5%. The trial, therefore, recruited a total of 5,000 women, 2,500 in each arm. Each of the four sites contributed 1,250 women recruited within an average of 16 months, to the trial sample.

3.3 Sample size calculations for the sub-studies

Objective 1: Determination of the accuracy of the First Response® Combo RDT for detecting placental malaria.

Placentas were examined histologically for all the women whose samples were available. We estimated that a minimum of 50% of all the women in the trial would have their placental samples processed and examined. Using the histological diagnosis of malaria as the end-point, the sensitivity and specificity of the RDTs in diagnosing placental malaria was estimated. We also calculated the predictive power of positive and negative First Response® Combo tests in diagnosing placental malaria.

Sample size calculation

A recent study conducted in Uganda obtained a prevalence of placental malaria by histology of 21.3%. The sensitivity of the RDT they used to detect placental malaria was 80.9% with a specificity of 87.5% (Kyabayinze et al. 2011). Assuming that the sensitivity of RDTs for detecting placental malaria is 81% with a specificity of 87% and that the prevalence of placental malaria is 21%, the sample size required to estimate the sensitivity of the RDT used in detecting placental malaria at 95% confidence interval of +/- 5% is 1,182 women.

Objective 2: Determination of the accuracy of the First Response® Combo RDT for detecting sub-microscopic peripheral malaria parasitaemia in pregnancy

This analysis was restricted to the women enrolled at the Ghana site only. All women in the intermittent screening and testing (IST) arm of the study had rapid diagnostic tests done at each visit. A total of 652 women in the IST arm had RDT testing at enrolment, decreasing to 564 at visit 1, 447 at visit 2 and 37 at visit 3 (refer table 5 for study visits). At delivery 379 women were tested. In addition, 191 women reported ill once. This number included 94 women in the IPTp arm of the study who also had RDTs done when they reported ill. Thirty women reported ill a second time, 15 of them belonging to the IPTp arm. Four women reported ill a third time, two of them from the IPTp arm. PCR assays were done on all blood spots collected at each study visit. A total of 4,100 blood spots obtained from women in both arms of the study were tested. These were made up of samples from all the five scheduled study visits [visits 0, 1, 2, 3 and 4 (delivery)] as well as unscheduled visits. The PCR assay results were used as the gold standard to assess the sensitivity and specificity of RDT to detect peripheral sub-microscopic parasitaemia.

Sample size calculation

A recent study by Kyabayinze et al. (2011) in Uganda obtained a sensitivity of 97% and a specificity of 74% for an HRP 2-type RDT for the diagnosis of MiP. Assuming that the level of sensitivity and specificity of the RDT for detecting malaria in pregnancy is similar, and the prevalence of submicroscopic parasitaemia will be 30%, to estimate the specificity of the RDT with a 95% confidence interval of +/- 5% a sample size of 990 women will be required. This same sample size will be sufficient to estimate a sensitivity of 97% with a 95% confidence limits of +/- 2%.

Objective 3: Determination of the prevalence and effect of submicroscopic malaria infections on pregnancy outcomes in pauci-gravid pregnant women.

We conducted PCR assays on all the blood spot samples collected from the women at one site (Ghana) of the trial. These included samples collected at both scheduled and unscheduled visits. Owing to the different gestational ages at which they were recruited, some women had made four ANC visits whiles others had made three or two visits before they delivered. The thick and thin blood films taken from this cohort at all the visits, both scheduled and unscheduled, until delivery were examined. PCR assays were done for all the blood spots from each visit for this cohort to determine the prevalence of submicroscopic infections among the cohort. The risk of placental malaria among women who had submicroscopic malaria during pregnancy but no RDT positive or blood slide positive malaria during ANC visits was estimated. The comparison of risk of other adverse outcomes (low birth weight and anaemia in pregnancy) was done for women who had submicroscopic infection during pregnancy, versus women who had microscopic malaria infections and those who had no malaria.

Sample size calculation

Several studies have obtained a prevalence of low birth weight among women who had placental malaria of around 20% (Steketee et al. 1996; McGregor et al. 1983). We assumed an incidence of low birth weight of 10% among women who had no malaria and 20% among women with submicroscopic malaria infections. To have 80% power and 95% significance to detect a difference in low birth weight between the two groups, a sample size of 392 women will be required.

Objective 4: Determination of the prevalence of falciparum and non-falciparum malaria in pregnant women in West Africa

A total of 2,348 samples blood spot samples were obtained from study participants at enrolment before any interventions began, from all the four countries involved in the trial. The distribution of the samples by country is as follows in Table 2:

Table 2 Distribution of women in sub-study by study country

Country	**Number (%)**
Burkina Faso	476 (20.3)
Gambia	725 (30.9)
Ghana	683 (29.0)
Mali	464 (19.8)
Total	**2,348 (100.0)**

Blood spot samples obtained from women were tested with a rapid diagnostic test for malaria, [First Response® Combo (pLDH/HRP2)]. Thick and thin blood films were prepared and filter paper samples for Nested PCR assays were also collected. The blood slides were examined twice by two microscopists at each site and if there were discrepancies in the results, slides were read by a third microscopist as described by Swysen et al (2011). For quality control, a 10% random sample of slides was selected and read by microscopists at an independent, designated centre. Speciation PCR assays were conducted at the MRC Unit laboratories in the Gambia.

Table 3: Sample size calculation scenarios for non-falciparum study

Parameters	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Estimated prevalence	0.01	0.02	0.03	0.04	0.05
Margin of error	0.01	0.01	0.01	0.01	0.01
Sample size	**381**	**753**	**1,118**	**1,476**	**1,825**

It is estimated that the prevalence of non-falciparum in this population is likely to be around 3%. This is supported by the findings of Mockenhaupt et al. (2000) who obtained a prevalence of 5% for *P. malariae* and 3.6% *for P. ovale* among a group of pregnant women in Ghana. Therefore, to estimate the prevalence of non-falciparum malaria among pregnant women with a 95% confidence interval within a margin of error of 1% required a sample size of 1,118 women. This needed to increase to 1,825 women if the prevalence was 5% (Table 3). Therefore, testing 2,500 women gave greater precision for this estimate. Owing to the fact that the women were recruited into the study over a whole year, we were able to disaggregate their data by season and also by place of recruitment.

Objective 5: Assessing the usefulness of a symptom and sign based score to predict MiP which could be used as a tool to select women needing RDT screening at antenatal clinics.

At each visit (scheduled and unscheduled), signs and symptoms were elicited from each pregnant woman. Specific questions about whether the woman had any symptoms on that day or whether she had had any symptoms in the past week were asked. The presence of particular symptoms such as fever, headache, and malaise was compared with the RDT test results obtained at those visits to determine if particular symptoms could predict the need for RDT testing at each visit. The predictive values of different combinations of symptoms and signs was also tested in RDT positive women compared to RDT negative women and in PCR positive vs. PCR negative women.

Sample size calculation

An earlier study in Ghana found that among a group of primigravidae, a history of sickness in the preceding five days before a clinic visit was predictive of parasitaemia in 57% of the women while a history of fever predicted parasitaemia in 44% of the women (Tagbor et al. 2008). We assumed therefore, that the sensitivity of clinical features to detect malaria in pregnancy was 50%, with a specificity of 50%. The sample size required to estimate the sensitivity of clinical features to detect MiP with a 95% confidence interval +/- 5% was 1,281.

3.4 Study Sites

3.4.1 Burkina Faso; health district of Ziniaré (Province of Oubritenga)

The Burkinabe component of the study was conducted in the health district of Ziniaré which is situated in the province of Oubritenga north of Ouagadougou, in Burkina Faso. The total population of the district was 261,892 in 2009 and the adult female population was 61,466. Twelve thousand pregnancies were expected per year. Child mortality fluctuates between 23 and 28 per 1000 live births per year. The district has one district hospital (35 km from Ouagadougou) and 41 health centres/dispensaries. The climate of the area is characteristic of the Sudan savannah with a dry, cool season from November to February, a dry, hot season from March to May and a rainy season from June to October. The mean annual rainfall is about 650 mm and the average annual temperature ranges between 23º and 33°C. Malaria is endemic and is the first cause of consultation and hospitalization. The trial was conducted by the UFR / SDS (School of Medicine), University of Ouagadougou with some support from the Centre National de Recherche et de Formation sur le Paludisme (CNRFP). This site has expertise in conducting trials in malaria in pregnancy, malaria epidemiology and drug resistance. Recent studies include a treatment trial comparing SP and

chloroquine and a study of intestinal parasite in pregnancy (Coulibaly et al. 2006).

3.4.2 Ghana: Navrongo Health Research Centre, Navrongo.

The Ghanaian component of the study was conducted in the Kassena-Nankana district of northern Ghana, which lies within the Sudan savannah belt of West Africa. Navrongo, the district capital, has an annual rainfall of 850-950mm, most of which falls between May and September. The district has a population of about 150,000 and the economy is dominated by subsistence farming. Educational attainment is generally low. The district has 4 health centres, 3 clinics and a 140-bed hospital that serves as a referral centre. All these facilities provide antenatal care and have facilities for delivery. These are complemented by services provided by community-based community health officers who offer ante-natal services. Trained midwives undertake deliveries as well. The district serves as a national HIV sentinel site. The trial was based at the Navrongo Health Research Centre, a well-equipped centre which has extensive experience with large field trials, including trials of ITNs and meningococcal and rotavirus vaccines. A large trial of IPTp with amodiaquine and SP + amodiaquine was recently completed which involved over 3600 pregnant women (Clerk et al. 2008; Clerk et al. 2009).

3.4.3 Mali: San and Kita field sites

The study in Mali was based at the community health centres of two medium sized, semi-rural towns (San and Kita) in north-eastern Mali, with a total population of approximately 75,000 inhabitants. San is located 440 kilometres northeast of Bamako in the Segou region of Mali; Kita is 140 km north-west of Bamako. Residents of both towns are of relatively similar socio-economic status. These areas have a climate typical of the Sudan savannah with a long dry season and a shorter rainy season when most malaria transmission occurs, extending from June to August (San) or September (Kita). Staff of the Malaria Research and Training Centre, Bamako were responsible

for conduct of the trial. This well-equipped centre has extensive experience of malaria research including the conduct of treatment and vaccine trials. MRTC is involved in all aspects of research on malaria and has an excellent track record for running complex field and intervention studies (Kayentao et al. 2005).

3.4.4 The Gambia: MRC Laboratories, Basse

The trial was conducted in the Upper River Division. The study area comprised Basse town and the surrounding rural areas. A demographic surveillance system is in place covering a population of approximately 120,000. The climate of the area is typical of the Sudan savanna with an annual rainfall of around 700 mm a year, which falls predominantly during the months of July – November, a little later that in the other sites in this trial. *P. falciparum* is the dominant malaria parasite. The study area is served by Basse Health Centre and by several dispensaries and health centres. Basse Health Centre has in-patient facilities for 100 patients. It has laboratory facilities for haematological, biochemical and parasitological investigations and X-ray facilities. Basse health centre is supported by the well-equipped MRC field station in Basse town. MRC is currently conducting surveillance studies on the causes of diarrhoea and acute respiratory infections in Upper River Division. Studies on the prevention of malaria in children using vaccination and intermittent preventive treatment have previously been conducted successfully in Upper River Division.

Table 4: Background information on the study sites or nearby areas

Parameter	Burkina Faso	Ghana	Mali	The Gambia
(1) EIR (bites/person/year)	100 – 300	200	19.23 – 21.1 during rainy season	10 – 50
(2) Malaria prevalence				
Children <5 years	53.5%	41.5%	39.5%	20%
a) Antenatal				
All gravidae	20 – 25%	47%	23.9%	8%
Primigravidae	25%	58%	62.1%	28%
b) Placenta				
All gravidae	22%	32%	17.1 – 42.3%	No recent data
Primigravidae			8.8%	
(3) ANC attendance	>90%	98%	>90%	98%
(4) SP-IPTp2 or more coverage	Not available	35%	4%	32%
(5) Antimalarial drug policy				
1st line	Quinine	Quinine	Quinine	Quinine
2nd line	AQ/AS; AL	AQ/AS; AL	AQ/AS; AL	AQ/AS; AL
(6) Folic acid supplementation dose	0.25mg	5mg	5mg	5mg
(7) Iron supplementation dose	200mg	200mg	200mg	200mg
(8) HIV control policy				
HIV prevalence among pregnant women	1.6%	2.8%	Low	2.8%
HIV screening at ANC	VCT	VCT	VCT	VCT
ART policy	Refer to ART centre for drugs	Refer to ART centre for drugs	Refer to ART centre for drugs	Refer to ART centre for drugs
(9) ITN coverage / use among pregnant women	Not available	40 – 52.5%	29%	50%

3.5 Study population

The trial considered for recruitment all primigravidae and secundigravidae who reported to an ANC within the study areas for the first time during that current pregnancy from the time the trial started until the required sample size was reached.

3.5.1 Inclusion criteria

1. Presence of a first or second pregnancy.
2. Gestational age between 16 to 30 weeks inclusive at first booking, as determined by symphysio-fundal measurements.
3. Provision of written informed consent to join the trial.
4. Residence in the study area and intention to stay in the area for the duration of the pregnancy.

3.5.2 Exclusion criteria

1. Absence of informed consent.
2. An intention to leave the study area before delivery.
3. A history of sensitivity to sulphonamides.
4. Clinical AIDS or known HIV positivity.
5. Presence of any systemic illness likely to interfere with interpretation of the results of the trial.

3.6 Study procedures

All study participants were recruited at ANCs at the various study sites over a one year period. They were all followed-up at scheduled visits till six weeks after they had delivered when they exited from the study. Table 5 below shows the schedule of visits and procedures conducted for each study participant.

Table 5: A schedule of procedures at study visits

STUDY PROCEDURE	SCHEDULE OF FOLLOW-UP VISITS						
	Visit 0 (enrolment day)	Visit 1 (24-26 weeks)	Visit 2 (32 weeks)	Visit 3 (38 weeks +/- 2 weeks)	Visit 4 (delivery)	Visit 5 (postpartum)	Unscheduled visits
Assess woman's eligibility	X						
Obtain informed consent	X						
Randomise eligible women into study arms	X						
Obtain demographic, medical and obstetric data (including history and physical examination)	X						
Symptoms assessment	X	X	X	X	X	X	X
Rapid diagnostic testing for malaria & treatment*	X	X	X		X	X	X
Provide SP IPTp^	X	X	X				
Obtain thin and thick blood smears for microscopy	X	X	X	X	X	X	X
Prepare filter paper blood spots	X	X	X	X	X	X	X
Assess Hb level	X			X	X		
Assess incidence adverse drug events		X	X	X	X		X
Collect placenta biopsies for impression smears and histology					X		
Record birth weight					X		
Assess gestational age using Ballard score					X		
Examine newborn and record any congenital abnormalities					X		
Assess neonatal and maternal outcomes						X	

* All women in intermittent screening and treatment arm and any woman at unscheduled visit
^All women in IPT arm

3.7 Symptomatology

A short questionnaire was administered to all women in the study to collect background information. Two short checklists were used to elicit symptoms and signs from women attending antenatal clinics. One of the checklists sought to obtain information on whether the woman had any symptoms at the time of being interviewed. The second checklist elicited symptoms which may have been present in the last seven days before the visit. This information was collected at every visit (scheduled and unscheduled). The relationship between these clinical features and a positive malaria RDT result were explored.

3.8 Laboratory procedures

3.8.1 Haemoglobin measurement

- Haemoglobin concentrations was measured from finger prick blood samples using an Hb 301 Hemocue™ analysers (HemoCue, Anglom, Sweden). Measurements were done three times for each woman; first at recruitment, then at 38 weeks (+/-2 weeks) and at the time of delivery or shortly afterwards. The tests were performed according to the manufacturer's instructions. Anaemia is defined as a haemoglobin concentration <11.0gm/dl (Goonewardene et al. 2012).

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3.8.2 Blood film

Thin and thick blood smears were obtained from all women on presentation (visit 0), at two subsequent visits when the project interventions were delivered (visits 1 and 2), at 38 weeks (+/- 2 weeks) [visit 3], at delivery (visit 4) and post-partum (visit 5). In addition, blood films were made at any time that a study woman presented at an unscheduled visit with suspected malaria as assessed by a member of the routine ANC clinic staff. Thick blood smears were stained with 2% Giemsa for 30 minutes and read by two trained microscopists independently

at each site. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes if the count was <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μl. A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites. A third microscopist read each slide for which there was a discrepancy between the first two readers. For quality control, a 10% random sample of slides was read by microscopists from a designated referral centre in Kenya. These results were used to monitor the slide reading of individual microscopists and to retrain those who needed it.

A limitation of the method for the quantification of malaria parasitaemia, which is based on an assumption of a white blood cell (WBC) count of 8000/microliter is the physiological leokocytosis that is observed in pregnancy (Sanci et al. 2017). This has the potential of resulting in a lower malaria parasite count than expected as there are more WBCs to count and therefore 8000 WBCs are more quickly counted. This may limit the usefulness of this method of counting malaria parasites. If an accurate WBC count is known, a more accurate parasite count could be obtained (Moody, 2002).

3.8.3 Rapid diagnostic tests (RDTs)

First Response® Malaria Rapid Diagnostic Combo Test was used in this trial. The same RDT was used at all sites. Members of recruitment teams received hands-on instruction from local investigators in preparation and interpretation of the RDTs according to the manufacturer's instructions. The test results from these RDTs were compared with PCR results for the same blood samples.

The First Response® Malaria pLDH/HRP2 combo Test contains a nitrocellulose membrane strip which is pre-coated with two monoclonal antibodies in two separate lines across a test strip. One monoclonal antibody (test line 2) is specific to lactate

dehydrogenase (pLDH) of the Plasmodium species (*P. falciparum, P.vivax, P.malariae,* and *P.ovale*) and the other line (test line 1) consists of a monoclonal antibody specific to Histidine-Rich Protein 2 (HRP2) of the *P. falciparum*.

The test is performed by taking a 5µl sample of blood with a calibrated sample pipette from a finger prick. The blood is dispensed into a sample well on the test cassette after which 60µl of assay buffer is added. The test result is read after 20 minutes. The presence of three bands on the cassette (control, test lines 1 & 2) indicates a positive test for *P. falciparum* or a mixed infection of *P. falciparum* and other non-falciparum species. The presence of two bands (control and test line 2) indicates a positive result for pan-plasmodium species possibly including *P. falciparum*. However, one would have expected the test line 1 to also be reactive if *P. falciparum* was to be present, unless for some reason, the HRP2 test strip gave a false negative result or the parasite did not produce HRP2. The presence of only the control band indicates a negative result. The test is invalid if the control band does not appear, irrespective of what the other bands show.

3.8.4 Placental malaria: Preparation of placental smears and biopsies

Immediately after delivery in a health facility, trained midwives obtained and washed placental samples with sterile normal saline. A small piece of placental tissue (0.5 cm^3) was excised from the centre of the maternal side of the placenta to prepare impression smears. A larger placenta biopsy (1.5 by 1.5 by 1 cm) was obtained and fixed in 50 ml of 10% neutral buffered formalin, adjusting the pH at 7.0, and individually labelled to help identification. Pending histological processing and evaluation, all biopsies were kept at 4 °C. The placental biopsies were embedded in paraffin wax by standard techniques in histopathology laboratories in each of the participating countries. In Ghana, the histopathological processing of the placenta blocks was done at the Histopathological Laboratory at the Korle Bu Teaching Hospital in Accra. Paraffin sections 4 µm thick were stained with hematoxylin and eosin (H&E) stain for

histopathological examination at the Medical Research Council (MRC) Unit laboratories in the Gambia by Ms. Fanta Njie who had been trained in this technique at the University of Barcelona. This allowed comparison of RDTs, microscopy, PCR and placental histology to be done.

The classification of placental malaria used in this study is that of Bulmer et al. (1993) as modified by Ismail et al. (2000).

For the purpose of these analyses, the definition of placental malaria was limited to active infection, comprising only those who had malaria parasites present in their placenta at the time of delivery. Past infections, characterised by the presence of malaria pigment but with parasites absent, were classified with those with neither pigment nor parasites as cases of 'no placental malaria'.

3.8.5 PCR

Nested PCR assays with speciation were conducted on blood samples collected on filter paper from all the women in the IST arm of the study in all the four countries (n=2,500), to determine the prevalence of non-falciparum malaria among the women. PCR was also done to detect submicroscopic infections and to compare the sensitivity and specificity of RDTs and microscopy in the diagnosis of malaria in pregnancy. PCR testing was conducted at the MRC Unit laboratories in the Gambia according to their SOPs (annex 1). In preparation for the PCR assays, parasite DNA was extracted from dried blood spots collected at the various sites. A summary of the nested PCR assay is as follows:

i. The first stage of the procedure involves a PCR assay with the primers **rPLU5 + rPLU6**. The assay includes positive controls for each of the four species (*falciparum, vivax, ovale and malariae*) and negative control without DNA.

ii. The second PCR reaction is to detect the presence of *Plasmodium* infection using the genus-specific primers, **Plasmo-1 + Plasmo-2.**

iii. The third stage seeks to determine the specific species in positive samples by running four parallel PCR assays using the primers below:

P. falciparum: **rFAL 1 + rFAL 2**

P. malariae: **rMAL 1 + rMAL 2**

P. ovale: **rOVA 1 + rOVA 2**

P. vivax:**rVIV 1 + rVIV 2**

For further differentiation of *P. ovale* into the two distinct species *P. ovale curtisi* and *P. ovale wallikeri*, the *P. ovale* sp. Tryptophan-rich antigen (PoTRA) nested PCR assay (Sutherland et al., 2010) was used.

In this thesis I assume that the finding of a PCR positive result for malaria is equivalent to infection with the malaria parasite. This position was adopted owing to the fact that PCR has so far had the highest sensitivity and specificity compared to all the currently available tests for malaria. It has been shown to detect five parasites or less per microliter of blood (Snounou et al, 1993). However, it must be noted that even though its sensitivity and specificity are high, absolute figures (100%) have never been obtained in any testing regime. Accordingly, there will be some false positive test results meaning that PCR positive test results will not always be equivalent to infection. The risk of this is however, quite low.

The PhD candidate was based at the MRC Unit in the Gambia from October to November 2012 and also for two weeks in March 2013 to take part in the PCR analyses of filter paper samples obtained for testing in the trial.

3.9 Socio-economic data collection and analysis

The socio-economic status of study participants was defined using principal component analysis of durable household assets and amenities. The data for this analysis was obtained from study participants at enrolment using the "Demography form" (Form 10.2) found in Appendix 1. The principal component analysis was used to create five wealth quintiles among the women, who were categorised as:

Wealthiest
Wealthy
Medium
Poor
Poorest

The student investigator supervised data collection at the Ghana site of the main trial including household assets and durable amenities which was obtained at enrolment.

3.10 Data management plan

This study was conducted within the context of the trial of IPT with SP versus intermittent screening and treatment for malaria in pregnancy conducted in four West African countries. Data in this trial were captured using Teleform (Cardiff Software Inc., Vista CA) an optical character recognition-based data management system that scans paper case report forms (CRF) and exports data to a computer database. The same pre-printed CRFs were used to capture enrolment and follow up data at all trial centres. All CRFs were received, checked and registered by the data management teams. Once the data were captured by Teleform, at each site, they were stored in a local database and an anonymised copy submitted to the central database in London for further checks and cleaning. STATA (version 13) software was used for performing the main analysis of all the data captured.

3.11 Data analysis

The baseline characteristics of all the study women were summarised with descriptive statistics. All categorical variables were presented as percentages while continuous variables were presented as means. The sensitivity and specificity analysis was conducted using the "diagt" command, a utility on STATA which generates 2 X 2 tables as well as the sensitivity, specificity, positive and negative predictive values. The analysis of sub-microscopic infections involved three outcomes of interest; placental malaria incidence, low birth weight and anaemia prevalence at 38 weeks of gestation. For these outcomes, the 'per protocol' populations were used. In logistic regression modeling for risk factors of sub-microscopic infections certain variables were assessed 'a priori' for their effects on the outcomes of interest. These include the age group of the women, educational status, gravidity, season of enrolment, intervention group and socio-economic status. For the modeling of malaria prevalence, these same variables were assessed in addition to country of residence and religion.

3.12 Ethical considerations

The main study protocol and its amendments were approved by the London School of Hygiene and Tropical Medicine Ethical Committee (Application number 5275). In Ghana, approvals were granted by the Ghana Health Service Ethical Review Committee and the Navrongo Health Research Centre Institutional Review Board; in Burkina Faso ethical approval was granted by the Ethical Committee of the Ministry of Health; in The Gambia approval was granted by the Gambia Government/Medical Research Council Laboratories Joint Ethical Committee and in Mali the University of Bamako Ethical Committee reviewed and approved the study protocol and its amendments. Ethical approvals obtained for this trial are attached as appendices.

Informed consent was obtained from all eligible women at the antenatal clinics before they were enrolled into the study.

3.13 Definitions

- **Low birth weight**: the weight of a live singleton newborn baby of less than 2,500 grammes obtained within seven days of delivery.
- **Anaemia:** Haemoglobin concentration of less than 11.0g/dl (Goonewardene et al. 2012)
- **Season of enrolment**:
    - Early wet: June-August.
    - Late wet: September-November.
    - Early dry: December-February.
    - Late dry: March-May.

The season of enrolment was included in this PhD sub-study because malaria transmission in the four study areas is occurs mainly during the rainy season or shortly afterwards and is therefore highly seasonal. Accordingly, I tried to explore associations between the season of enrollment and some of my outcomes of interest. However, the categories used could reflect slightly different rainfall patterns in each of the countries. This potential misclassification is a limitation of this method of categorising rainfall seasons in the study countries.

- **Paucigravida(e):** In this thesis, this refers to women pregnant for the first or second time only.

CHAPTER FOUR

Sensitivity and Specificity of Rapid Diagnostic Tests (RDTs) in the Diagnosis of Malaria in Pregnancy

4.1 Introduction

This chapter presents the findings of analyses of the sensitivity and specificity of RDTs to detect malaria infection in the peripheral blood and placenta of Ghanaian pregnant women participating in a trial of IPT with sulfadoxine- pyrimethamine versus IST.

First the prevalence of malaria infection of the placenta and of a positive RDT test is reported. Then, the sensitivity and specificity of the RDT used in the study to detect placental malaria are presented. Subsequently, the sensitivity and specificity of the RDT are estimated for individual study visits. The sensitivity and specificity of the RDT to detect malaria infection in peripheral blood compared with PCR and blood smear microscopy is then presented.

4.2 Diagnosis of placental malaria by RDT

4.2.1 Prevalence of placental malaria

Table 6 shows the prevalence of malaria infection in 851 Ghanaian women whose placentae were examined histologically. Eight percent had acute placental malaria (malaria parasites without pigment) while 21.9% had chronic placental malaria (parasites and pigment), a total of 30.1% active placental malaria infections according to our definition (presence of malaria parasites with or without pigment and/or macrophages in the placenta) at the time of delivery. Even though past malaria infections are important clinically, they cannot be detected by RDT. Therefore, in assessing RDT sensitivity, active malaria infections where parasites are present with or without pigment is of interest.

Table 6: Distribution of active placental malaria among study participants

Characteristic	Number	Percent
No placental malaria	595	69.9
Active placental malaria		
Acute	70	8.2
Chronic	186	21.9
Total	**851**	**100.0**

4.2.2 Prevalence of malaria by Rapid Diagnostic Testing

The study participants who were in the IST arm of the study had scheduled rapid diagnostic testing done whenever they visited an ANC for a routine scheduled visit. Accordingly, they were tested at visits 0 (day of enrolment), 1, 2, 3 and 4 (delivery). In addition, any study subject irrespective of study arm, who reported sick at a scheduled or unscheduled visit also had a malaria rapid diagnostic test done. Two composite groups of women were created to estimate the prevalence RDT positive malaria in pregnancy. One group comprised women who had ever tested positive with an RDT at any time during the study, at a scheduled or an unscheduled visit (Ever RDT Positive), and the other group comprised women who had tested positive at any scheduled ANC visit (Any ANC RDT Positive). The results this analysis are presented in table 7 below. Nearly three quarters of women (73.5%) had at least one positive RDT during scheduled ANC visits. The majority of women had a positive RDT at the first ANC visit (58.3%) and the rate of RDT positivity decreased in the subsequent ANC visits, the last ANC visit had the lowest positive RDT (23.5%)

Table 7 Results of Rapid Diagnostic Tests

Characteristic	Number	Percent
Ever RDT positive		
No	321	34.8
Yes	601	65.2
Any ANC RDT Positive		
No	177	26.5
Yes	490	73.5
RDT Visit 0		
Negative	272	41.7
Positive	380	58.3
RDT Visit 1		
Negative	366	64.9
Positive	198	35.1
RDT Visit 2		
Negative	308	68.9
Positive	139	31.1
RDT Visit 3		
Negative	23	62.2
Positive	14	37.8
RDT Visit 4		
Negative	290	76.5
Positive	89	23.5
First illness RDT		
Negative	81	42.4
Positive	110	57.6
Second illness RDT		
Negative	14	46.7
Positive	16	53.3

4.2.3 Sensitivity and specificity of RDTs in detecting placental malaria.

Prevalence of active placental malaria was 30.0% (95% CI 27.0%, 34.2%). The number of RDT done per women during antenatal period ranged from 1 to 7. Six hundred and forty women had at least one RDT and had a placental histology result. In this group of women (Ever RDT positive group) the sensitivity of RDT to detect active placental malaria was 63.1% (95% CI 55.9%- 69.9%) and the Specificity was 35.1% (95% CI 30.6%- 39.7%) (Table 8). The positive predictive value of Ever RDT Positive was 29.9% (95% CI 25.5%- 34.5%) and the negative predictive value was 68.4% (95% CI 62.0%- 74.4%).

Table 8: Ever RDT Positive versus Active Placental Malaria

Ever RDT Positive	**Active Placental Malaria**		
	Positive	**Negative**	**Total**
Positive	123	289	412
Negative	72	156	228
Total	195	445	640

Four hundred and thirty-seven women had a RDT at scheduled ANC visit (Any ANC RDT) and a placental histology result. Sensitivity of Any ANC Positive RDT to detect placental malaria was 72.0% (95% CI 63.3%- 79.7%) and specificity was 25.3% 95% CI 20.6%- 30.5%) (Table 9). The positive predictive value was 27.9% (95% CI 23.0%- 33.1%) and negative predictive value was 69.3% (95% CI 60.0%- 77.6%).

Table 9: Any ANC Positive RDT versus Active Placental Malaria

Any ANC Positive RDT	**Active Placental Malaria**		
	Positive	**Negative**	**Total**
Positive	90	233	323
Negative	35	79	114
Total	125	312	437

Among the 424 women who had a RDT done at enrolment and had a placental histology result, the sensitivity of RDT to detect placental malaria was 55.7% (95% CI 46.5%- 64.7%) and specificity was 42.4% (95% CI 36.7%- 48.2%) (Table 10). The positive predictive value

was 28.1% (95% CI 22.5%- 34.2%) and negative predictive value is 70.3% (95% CI 63.1%- 76.9%)

Table 10: ANC Visit 0 RDT versus Active Placental Malaria

RDT visit 0	Active Placental Malaria		
	Positive	**Negative**	**Total**
Positive	68	174	242
Negative	54	128	182
Total	122	302	424

Among the 378 women who had a RDT done at ANC visit 1 post enrolment and had a placental histology result, the sensitivity of the RDT to detect placental malaria was 39.1% (95% CI 29.9%- 48.9%) and the specificity was 66.8% (95% CI 60.8%- 72.4%) (Table 11). The positive predictive value was 32.6% (95% CI 24.7%- 41.3%) and negative predictive value was72.8% (95% CI 66.7%- 78.2%).

Table 11: Visit 1 RDT versus Active Placental Malaria

RDT visit 1	Active Placental Malaria		
	Positive	**Negative**	**Total**
Positive	43	89	132
Negative	67	179	246
Total	110	268	378

Among the 310 women who had a RDT done at ANC visit 2 post enrolment and had a placental histology result, the sensitivity to detect active placental malaria was 33.3% (95% CI 23.4%- 44.5%) and the specificity was 71.2% (95% CI 64.9%- 77.0%) (Table 12). The positive predictive value was 30.1% (95% CI 21.0%- 40.5%) and negative predictive value was 74.2% (95% CI 67.8%- 79.9%).

Table 12: Visit 2 RDT versus Active Placental Malaria

RDT visit 2	Active Placental Malaria		
	Positive	**Negative**	**Total**
Positive	28	65	93
Negative	56	161	217
Total	84	226	310

Among the 317 women who had a RDT done at delivery and had a placental histology result,

the sensitivity of RDT to detect active placental malaria was 35.4% (95% CI 26.6%- 45.0%) and the specificity was 80.9% (95% CI 74.8%- 86.0%) (Table 13). The positive predictive value was 50.6% (95% CI 39.1%- 62.1%) and the negative predictive value was 69.3% (95% CI 63.0%- 75.1%).

Table 13: Visit 4 (delivery) RDT versus Active Placental Malaria

RDT visit 4	Active Placental Malaria		
	Positive	**Negative**	**Total**
Positive	40	39	79
Negative	73	165	238
Total	113	204	317

4.3 Diagnosis of malaria infection in pregnancy by RDT testing of peripheral blood.

This section presents the analyses of the sensitivity and specificity of the RDT when compared with PCR results obtained from testing of peripheral blood spots on filter paper or with blood smear microscopy. The analysis in this section is in reference to any malaria infection detected by PCR and not in reference to any particular species.

4.3.1 Sensitivity and specificity of the RDT in detecting PCR positive peripheral malaria parasitaemia.

Among 592 women who had a blood slide and PCR assay at enrolment the prevalence of PCR positive malaria was 57.0% (95% CI 53.0%-61.0%). The Sensitivity of RDT to detect PCR positive malaria was 89.3% (95% CI 85.5%-92.4%) and the specificity was 88.2% (95% CI 83.6%-91.9%) (Table 14). The positive predictive value was 90.9% (95% CI 87.3%-93.8%) and the negative predictive value was 86.2% (95% CI 81.4%-90.1%)

Table 14: Visit 0 RDT versus PCR results on a blood spot

V0 RDT Result	V0 PCR Result		
	Positive	**Negative**	**Total**
Positive	301	30	331
Negative	36	225	261
Total	337	255	592

Among 499 women who had a blood slide and PCR assay done at the visit 1 post enrolment the prevalence of PCR positive malaria was 23.0% (95% CI 19.0%-26.8%). The sensitivity of RDT to detect malaria was 85.1% (95% CI 77.2%-91.1%) and the specificity was 86.0% (95% CI 82.1%-89.3%) (Table 15). The positive predictive value was 64.2% (95% CI 56.0%-71.9%) and the negative predictive value was 95.1% (95% CI 92.3%-97.1%).

Table 15: Visit 1 RDT versus PCR results on a blood spot

V1 RDT Result	V1 PCR Result		
	Positive	**Negative**	**Total**
Positive	97	54	151
Negative	17	331	348
Total	114	385	499

Among 393 women who had a blood slide and PCR assay done at the visit 2 post enrolment the prevalence of PCR positive malaria was 18.0% (95% CI 15.0%-22.5%). The sensitivity of RDT to detect PCR positive malaria was 79.2% (95% CI 68.0%-87.8%) and the specificity was 82.9% (95% CI 78.3%-96.8%) (Table 16). The positive predictive value was 50.9% (95% CI 41.3%-60.5%) and negative predictive value was 94.7% (95% CI 91.4-97.0%)

Table 16: Visit 2 RDT versus PCR results on a blood spot

V2 RDT Result	V2 PCR Result		
	Positive	**Negative**	**Total**
Positive	57	55	112
Negative	15	266	281
Total	72	321	393

Among 348 women who had a blood slide and PCR assay done at delivery the prevalence of PCR positive malaria was 15.0% (95% CI 11.0%-19.1%). The sensitivity of RDT to detect PCR positive malaria was 57.7% (95% CI 43.2%-71.3%) and the specificity was 83.1 (95%

CI 78.3%-87.2%) (Table 17). The positive predictive value was 37.5% (95% CI 26.9%-49.0%) and the negative predictive value was 91.8% (95% CI 87.8%-94.8%).

Table 17: Visit 4 RDT versus PCR result on a blood spot

V4 RDT Result	V4 PCR Result		
	Positive	**Negative**	**Total**
Positive	30	50	80
Negative	22	246	268
Total	52	296	348

4.3.2 Sensitivity and specificity of RDT in detecting malaria parasitaemia as detected by microscopy

Among 651 women who had a RDT and blood slide at enrolment the prevalence of blood slide positive malaria was 47.0% (95% CI 43.0%-50.9%). The sensitivity of RDT to detect blood slide positive malaria was 95.8% (95% CI 92.8%-97.7%) and the specificity was 75.1% (95% CI 70.2%-79.5%) (Table 18). The positive predictive value was 77.3% (95% CI 72.8%-81.4%) and the negative predictive value was 95.2% (95% CI 92.0%-97.4%).

Table 18: Visit 0 RDT versus blood smear microscopy results

V0 RDT Result	V0 Blood Smear Result		
	Positive	**Negative**	**Total**
Positive	293	86	379
Negative	13	259	272
Total	306	345	651

Among 363 women who had a RDT and blood slide done at visit 1 post enrolment the prevalence of blood slide positive malaria was 22.0% (95% CI 19.0%-26.0%). The sensitivity of RDT to detect malaria in pregnancy was 91.2% (95% CI 84.4%-95.5%) and the specificity was 81.1% (95% CI 77.1%-84.7% (Table 19). The positive predictive value was 58.2% (95% CI 50.9%-65.2%) and the negative predictive value was 97.0% (95% CI 94.6%-98.5%).

Table 19: Visit 1 RDT versus blood smear microscopy result

V1 RDT Result	V1 Blood Smear Result		
	Positive	**Negative**	**Total**
Positive	114	82	196
Negative	11	352	363
Total	125	434	559

Among 438 women who had a RDT and blood slide done at visit 2 post enrolment the prevalence of blood slide positive malaria was 18.0% (95% CI 14.0%-21.5%). The

sensitivity of RDT to detect malaria in pregnancy was 92.2% (95% CI 83.8%-97.1%) and the specificity was 82.3% (95% CI 77.9%-86.1%) (Table 20). The positive predictive value was 52.6% (95% CI 43.8%-61.2%) and the negative predictive value was 98.0% (95% CI 95.7%-99.3%).

Table 20: Visit 2 RDT versus blood smear microscopy results

V2 RDT Result	V2 Blood Smear Result		
	Positive	**Negative**	**Total**
Positive	71	64	135
Negative	6	297	303
Total	77	361	438

Among 344 women who had a RDT and blood slide done delivery the prevalence blood slide positive malaria was 17.0% (95% CI 13.0%-21.2%). The sensitivity of RDT to detect malaria in pregnancy was 60.3% (95% CI 46.6%-73.0 %%) and the specificity was 85.0% (95% CI 80.3%-88.9%) (Table 21). The positive predictive value was 44.9% (95% CI 33.6%-56.6%) and the negative predictive value was 91.4% (95% CI 87.3%-94.4%).

Table 21: Visit 4 RDT versus blood smear microscopy results

V4 RDT Result	V4 Blood Smear Result		
	Positive	**Negative**	**Total**
Positive	35	43	78
Negative	23	243	266
Total	78	256	344

4.4 Summary

The sensitivity of any positive RDT test at scheduled and unscheduled visits to detect placental malaria was 63.1% (Ever RDT positive) and it increased to 72.0% when the RDT done at scheduled visits alone were used (ANC RDT positive). However, the specificity of the RDT to detect placental malaria was much lower as it ranged between 25.3% for Any RDT Positive to 35.1% for Ever RDT Positive. The sensitivity of the RDT to detect placental malaria at individual study visits ranged between 55.7% at the enrolment to 33.3% during the 2nd visit post enrolment. At the same time, the specificity of the test result ranged from a low of 42.4% at enrolment to a high of 80.9% during the delivery visit. The sensitivity of the RDT to detect PCR positivity in peripheral blood ranged between 57.7% at delivery to 89.3% at enrolment and specificity ranged between 82.9% obtained during 2nd visit post enrolment to 88.2% at enrolment. When blood smear microscopy was used as the gold standard, the sensitivity of the RDT to detect malaria in pregnancy ranged between 60.3% for the delivery visit to 95.8% at enrolment. The specificity ranged between 75.1% at enrolment to 85.0% at delivery.

Table 22: Summary of RDT sensitivity and specificity with various reference tests

Reference test	RDT	N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
			% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Placental histology						
	Ever RDT Positive	640	63.1 (55.9-69.9)	35.1 (30.6-39.7)	29.9 (25.5-34.5)	68.4 (62.0-74.4)
	Any ANC Positive	437	72.0 (63.3-79.7)	25.3 (20.6-30.5)	27.9 (23.0-33.1)	69.3 (60.0-77.6)
	Visit 0	424	55.7 (46.5-64.7)	42.4 (36.7-48.2)	28.1 (22.5-34.2)	70.3 (63.1-76.9)
	Visit 1	378	39.1 (29.9-48.9)	66.8 (60.8-72.4)	32.6 (24.7-41.3)	72.8 (66.7-78.2)

	Visit 2	310	33.3 (23.4-44.5)	71.2 (64.9-77.0)	30.1 (21.0-40.5)	74.2 (67.8-79.9)
	Visit 4	317	35.4 (26.6-45.0)	80.9 (74.8-86.0)	50.6 (39.1-62.1)	69.3 (63.0-75.1)
Blood spot PCR						
	Visit 0	592	89.3 (85.5-92.4)	88.2 (83.6-91.9)	90.9 (87.3-93.8)	86.2 (81.4-90.1)
	Visit 1	499	85.1 (77.2-91.1)	86.0 (82.1-89.3)	64.2 (56.0-71.9)	95.1 (92.3-97.1)
	Visit2	393	79.2 (68.0-87.8)	82.9 (78.3-96.8)	50.9 (41.3-60.5)	94.7 (91.4-97.0)
	Visit 4	348	57.7 (43.2-71.3)	83.1 (78.3-87.2)	37.5 (26.9-49.0)	91.8 (87.8-94.8)
Microscopy						
	Visit 0	651	95.8 (92.8-97.7)	75.1 (70.2-79.5)	77.3 (72.8-81.4)	95.2 (92.0-97.4)
	Visit 1	559	91.2 (84.4-95.5)	81.1 (77.1-84.7)	58.2 (50.9-65.2)	97.0 (94.6-98.5)
	Visit 2	438	92.2 (83.8-97.1)	82.3 (77.9-86.1)	52.6 (43.8-61.2)	98.0 (95.7-99.3)
	Visit 4	344	60.3 (46.6-73.0)	85.0 (80.3-88.9)	44.9 (33.6-56.6)	91.4 (87.3-94.4)

PPV: positive predictive value, NPV: negative predictive value

In summary the RDT used in this study failed to detect a number of malaria infections of both peripheral blood and placenta compared to more sensitive assays (PCR and placental histology). The significance of these failures is considered in the next two chapters.

CHAPTER FIVE

The influence of sub-microscopic and RDT negative malaria infections on the outcome of pregnancy

5.1 Introduction

This chapter analyses the impact of malaria infections that were not detected by an RDT or by microscopy on the outcome of pregnancy in the Ghanaian women enrolled in a trial of IPTp-SP versus IST. The analyses include all the 1,306 women enrolled at the Ghana site into both the IPTp-SP and IST arms of the study.

In this analysis of sub-patent malaria infections, infections detected by PCR and not by RDT (classified sub-RDT infections) are presented first followed by infections detected by PCR but not by microscopy (sub-microscopic). The women were studied separately for the first three antenatal clinic visits when study interventions were being provided for those in the intervention arm (v0-v2) and also for all the visits including the delivery visit (v0-v4).

5.2 Background characteristics of study participants

Table 23 presents the background characteristics of participants in this part of the study. The mean age of the women in the sample was 21.6 years with a minimum of 14 years and a maximum of 37 years. Almost 80% of the women were aged less than 25 years old. More than one-third were housewives with a significant proportion of others being traders or farmers. A fifth of the women had received no formal education while 65% had received only basic education. A little more than half of the women in the sample were carrying their first pregnancy. Most of the women were poor, with 53% of them being within the lowest two levels of the socio-economic scale (poor and poorest).

Table 23: Background characteristics of study participants

Characteristic	Number	Percent
Age group (years)		
<20	443	33.9
20-24	590	45.2
25+	273	20.9
Occupation		
Housewife	485	37.1
Farmer	263	20.1
Trader	368	28.2
Salaried worker	76	5.8
Other	114	8.7
Education		
None	266	20.4
Basic	849	65.0
Secondary	191	14.6
Gravidity		
1	727	55.8
2	576	44.2
Socio-economic status		
Wealthiest	162	12.4
Wealthy	167	12.8
Medium	278	21.4
Poor	453	34.8
Poorest	242	18.6
Season of enrolment		
Early wet	456	34.9
Late wet	280	21.4
Early dry	225	17.2
Late dry	345	26.4
Total	**1,306**	**100.0**

5.3 Prevalence of sub-patent malaria infections

5.3.1 Sub-RDT malaria infections

Table 24 shows the distribution of RDT positive malaria and sub-RDT infections (RDT –ve but PCR +ve) detected at the first three scheduled ANC visits as well as at the delivery visit. This analysis is restricted to the women in the IST arm only, as only they had RDTs done at

scheduled visits. The table also shows the risk of sub-RDT infections calculated for the first three study visits (v0-v2) and also for all the study visits combined (v0-v4).

Table 24: Distribution of sub-RDT infections among women in IST arm by study visit

Study Visit	n	Percent
First visit (v0)	(N=592)	
Uninfected	225	38.0
RDT positive	331	55.9
Sub-RDT	36	6.1
Second visit (v1)	(N=499)	
Uninfected	331	66.3
RDT positive	151	30.3
Sub-RDT	17	3.4
Third visit (v2)	(N=393)	
Uninfected	266	67.7
RDT positive	112	28.5
Sub-RDT	15	3.8
Delivery visit (v4)	(N=348)	
Uninfected	246	70.7
RDT positive	80	23.0
Sub-RDT	22	6.3
Risk of infection (v0-v2)	(N=625)	
Uninfected	139	22.2
RDT positive	423	67.7
Sub-RDT	63	10.1
Risk of infection (v0-v4)	(N=636)	
Uninfected	123	19.3
RDT positive	427	67.1
Sub-RDT	86	13.5

At first ANC visit, 55.9% of women had malaria infections detected by RDT and PCR; an additional 6.1% had sub-RDT infections. At delivery, 23.0% of study women had RDT positive malaria infections which were also positive by PCR. However, there were an additional 6.3% of women who had sub-RDT infections.

The risk of a positive RDT from the first to the third scheduled (v0-v2) visit was 67.7% while the risk for additional sub-RDT malaria infection was 10.1%. However, when all the scheduled

visits including delivery are combined (v0-v4), the risk of a positive RDT was 67.1% while that of sub-RDT infections increased to 13.5%.

Table 25 shows the influence of various background characteristics on the prevalence of sub-RDT infections at the first two scheduled visits and also at the delivery visit. A trend towards increasing prevalence of sub-RDT infections with increasing age was observed at the first two visits. The prevalence increased from 4.0% (95% CI 1.9-8.1) among women aged less than 20 years to 7.8% (95% CI 4.2-13.9) among women 25 years and above during the first visit. During the second visit, the sub-RDT prevalence increased from 2.8% (95% CI 1.0-7.2) among the youngest women to 4.6% (95% CI 1.9-10.6) among the oldest women. This trend was not so clear during the delivery visit when the prevalence decreased from 6.1% (95% CI 3.1-11.8) among the women less than 20 years to 5.0% (95% CI 2.4-10.2) among the women 20-24 years and then increased to 9.1% (95% CI 4.4-18.0) among the oldest women 25 years and above.

From the second scheduled visit, there was a trend towards a decreasing prevalence of sub-RDT infection with increasing education of the women. The prevalence of sub-RDT infections decreased from 6.4% (95% CI 3.1-12.8) among women with no education to 2.2% (95% CI 0.5-8.3) among women with secondary education or more. A similar pattern was observed at delivery where sub-RDT infections decreased from 7.8% (95% CI 3.3-17.6) among women with no education to 4.3% (95% CI 1.1-15.8) among women with secondary education or more. However, it must be noted that the confidence intervals for this analysis overlap and therefore these observations may not be significant.

At the first two scheduled visits, secundigravid women had a higher prevalence of sub-RDT infections compared to primigravid women. However, at the delivery visit, this observation was reversed, with primigravid women having a higher prevalence of sub-RDT infections compared to secundigravid women. At their first visit, 9.6% (95% CI 6.6-13.7) of

secundigravid women had a sub-RDT malaria infection compared to 3.2% (95% CI 1.7-5.8) of primigravid women. At the delivery visit, the secundigravid women had a sub-RDT prevalence of 5.8% (95% CI 3.1-10.9) compared to 6.7% (95% CI 3.9-11.2) for the primigravid women.

There was no clear trend or pattern for the prevalence of sub-RDT infections by season of enrolment or socio-economic status of the women as observed in the three study visits analysed.

Table 25: Prevalence of sub-RDT malaria infection by background characteristics of study women at various visits

Characteristic	First visit (v0)		Second visit (v1)		Delivery visit (v4)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Age group (years)	(N=592)		(N=499)		(N=348)	
<20	7(4.0)	1.9-8.1	4(2.8)	1.0-7.2	8(6.1)	3.1-11.8
20-24	19(6.6)	4.3-10.2	8(3.3)	1.6-6.4	7(5.0)	2.4-10.2
25+	10(7.8)	4.2-13.9	5(4.6)	1.9-10.6	7(9.1)	4.4-18.0
Educational status	(N=592)		(N=499)		(N=348)	
None	10(7.5)	4.1-13.4	7(6.4)	3.1-12.8	5(7.8)	3.3-17.6
Basic	20(5.5)	3.6-8.4	8(2.7)	1.4-5.3	15(6.3)	3.8-10.3
Secondary	6(6.3)	2.9-13.4	2(2.2)	0.5-8.3	2(4.3)	1.1-15.8
Gravidity	(N=589)		(N=497)		(N=348)	
1	10(3.2)	1.7-5.8	7(2.6)	1.2-5.4	13(6.7)	3.9-11.2
2	26(9.6)	6.6-13.7	10(4.4)	2.4-8.0	9(5.8)	3.1-10.9
Season of enrolment	(N=592)		(N=499)		(N=348)	
Early wet	11(5.6)	3.1-9.9	4(2.8)	1.0-7.2	11(9.2)	5.2-16.0
Late wet	10(7.6)	4.1-13.7	5(3.9)	1.6-9.2	1(3.6)	0.5-22.2
Early dry	8(7.9)	3.8-14.3	2(2.1)	0.5-8.0	4(4.9)	1.9-12.5
Late dry	7(4.4)	2.1-9.0	6(4.6)	2.1-9.9	6(5.0)	2.3-10.7
Socio-economic status	(N=591)		(N=498)		(N=347)	
Wealthiest	5(6.2)	2.6-14.1	1(1.4)	0.2-9.6	5(12.2)	5.1-26.4
Wealthy	2(2.9)	0.7-11.1	4(7.1)	2.7-17.7	3(7.7)	2.5-21.6
Medium	6(4.8)	2.2-10.4	0(0.0)	-	6(8.0)	3.6-16.8
Poor	17(8.4)	5.3-13.1	9(4.9)	2.6-9.2	5(4.0)	1.7-9.3
Poorest	6(5.2)	2.4-11.2	3(3.3)	1.0-9.9	3(4.5)	1.4-13.1

5.3.2 Sub-microscopic malaria infections

At their first ANC visit, 47.9% of study women tested positive for malaria by microscopy as shown in table 26. However, 58.5% tested positive by PCR, indicating a sub-microscopic infection rate of 10.6%. The prevalence of sub-microscopic infections declined in subsequent

visits as the prevalence of malaria overall declined. The prevalence of sub-microscopic infections was 7.1% at the second visit, 5.8% at the third visit and 5.1% at delivery.

The overall risk of sub-microscopic infections for the first three scheduled ANC visits when interventions were provided was 17.6%. However, when all scheduled visits including delivery are combined, the risk of a sub-microscopic infection at any time within the pregnancy was 21.8%.

Table 26: Distribution of microscopic and sub-microscopic infections by study visit among study participants

Study Visit	N	Percent
First visit (v0)	(N=1,223)	
Uninfected	507	41.5
Microscopic	586	47.9
Sub-microscopic	130	10.6
Second visit (v1)	(N=1,014)	
Uninfected	730	72.0
Microscopic	212	20.9
Sub-microscopic	72	7.1
Third visit (v2)	(N=794)	
Uninfected	613	77.2
Microscopic	135	17.0
Sub-microscopic	46	5.8
Fourth visit (v3)	(N=506)	
Uninfected	421	83.2
Microscopic	61	12.1
Sub-microscopic	24	4.7
Delivery visit (v4)	(N=897)	
Uninfected	723	80.6
Microscopic	128	14.3
Sub-microscopic	46	5.1
Risk of infection (v0-v2)	(N=1,265)	
Uninfected	386	30.5
Microscopic	657	51.9
Sub-microscopic	222	17.6
Risk of infection (v0-v4)	(N=1,273)	

Uninfected	313	24.6
Microscopic	682	53.6
Sub-microscopic	278	21.8

Table 27 shows the influence of various background characteristics on the prevalence of sub-microscopic infections at the three ANC and delivery visits. There were no clear trends or patterns influencing the prevalence of sub-microscopic infections according to age group, educational status, intervention arm, season of enrolment or socio-economic status. However, secundigravid women had a higher prevalence of sub-microscopic malaria infection compared to primigravid women at all the three ANC visits. At the first visit, 12.4% (95% CI 9.9-15.4) of secundigravid women had sub-microscopic infections compared to 9.24% (95% CI 7.3-11.7) of primigravid women. However, at delivery this difference had largely disappeared and 5.4% (95% CI 3.5-8.1) of secundigravid women had sub-microscopic malaria infections compared to 5.0% (95% CI 3.4-7.2) of primigravid women. There was no difference in the prevalence of sub-microscopic infection in women in the two study arms. At first visit, 9.1% (95% CI 7.0-11.6) of women in the IPT arm had sub-microscopic infections compared to 12.3% (95% CI 9.9-15.2) of women in the IST arm. However, at second visit, women in the IST arm had a lower prevalence of sub-microscopic infections [6.3% (95% CI 4.5-8.8) vs. 7.9% (95% CI 5.8-10.5)]. At delivery, the prevalence of sub-microscopic infections was similar; women in the IPT arm had a prevalence of 5.0% (95% CI 3.4-7.4) while those in the IST arm had a prevalence of 5.3% (95% CI 3.5-7.8).

Table 27: Prevalence of sub-microscopic malaria infection by background characteristics of study women at various visits

Characteristic	First visit (v0)		Second visit (v1)		Delivery visit (v4)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Age Group (years)	(N=1,223)		(N=1,014)		(N=897)	
<20	35(8.6)	6.3-11.8	23(6.9)	4.6-10.1	15(4.9)	2.9-7.9
20-24	65(11.6)	9.2-14.6	38(8.3)	6.1-11.2	20(5.0)	3.3-7.7
25+	30(11.6)	8.2-16.1	11(5.0)	2.8-8.8	11(5.8)	3.2-10.2
Educational status	(N=1,223)		(N=1,014)		(N=897)	
None	26(10.4)	7.1-14.8	19(9.2)	5.9-14.0	13(7.3)	4.3-12.1
Basic	84(10.7)	8.7-13.1	46(7.2)	5.5-9.5	23(4.0)	2.7-6.0
Secondary	20(10.6)	7.0-15.9	7(4.1)	2.0-8.4	10(7.0)	3.8-12.5
Intervention Arm	(N=1,223)		(N=1,014)		(N=897)	
IPT	57(9.1)	7.0-11.6	41(7.9)	5.8-10.5	23(5.0)	3.4-7.4
IST	73(12.3)	9.9-15.2	31(6.3)	4.5-8.8	23(5.3)	3.5-7.8
Gravidity	(N=1,220)		(N=1,012)		(N=896)	
1	62(9.2)	7.3-11.7	35(6.4)	4.6-8.7	25(5.0)	3.4-7.2
2	68(12.4)	9.9-15.4	37(8.0)	5.9-10.9	21(5.4)	3.5-8.1
Season of enrolment	(N=1,223)		(N=1,014)		(N=897)	
Early wet	32(7.8)	5.6-10.8	27(8.4)	5.8-12.0	21(7.5)	5.0-11.3
Late wet	41(15.3)	11.5-20.1	19(7.8)	5.0-11.9	12(5.5)	3.1-9.4
Early dry	26(12.0)	8.3-17.1	7(3.8)	1.8-7.7	7(4.1)	2.0-8.4
Late dry	31(9.4)	6.7-13.1	19(7.2)	4.6-11.0	6(2.6)	1.2-5.8
Socio-economic status	(N=1,220)		(N=1,011)		(N=894)	
Wealthiest	18(11.5)	7.4-17.6	6(4.7)	2.1-10.1	7(5.5)	2.6-11.2
Wealthy	14(8.9)	5.3-14.5	2(1.5)	0.4-5.9	5(4.4)	1.8-10.2
Medium	22(8.2)	5.5-12.2	17(8.3)	5.2-12.9	10(5.4)	2.9-9.8
Poor	54(13.0)	10.1-16.6	27(7.3)	5.1-10.5	14(4.5)	2.7-7.4
Poorest	22(9.9)	6.6-14.6	20(11.4)	7.5-17.1	10(6.5)	3.5-11.7

5.4 Predictors of sub-patent malaria infections

A number of factors were evaluated for their association with sub-patent malaria infections among the study women at any stage of their pregnancy. For sub-RDT infections, the same factors with the exception of intervention arm were assessed. The intervention arm was not assessed because RDTs were conducted routinely only for women in the IST arm and not the ITPp-SP arm. For sub-microscopic infections, the age group, educational status, intervention arm, gravidity, season of enrolment and socio-economic status of the study women were assessed for their ability to predict sub-microscopic infections.

5.4.1 Predictors of sub-RDT malaria infections

Table 28 shows the influence of various background characteristics on the prevalence of sub-RDT malaria estimated for the first three visits as well as for all the visits combined.

There was a trend of increasing prevalence of sub-RDT infection with increasing age group. Women who were less than 20 years old had the lowest prevalence of sub-RDT infection (7.9%, 95% CI 4.8-12.6) which increased to 11.0% (95% CI 6.7-17.4) among women 25 years and above during the first three visits. The same trend was observed when all the visits are combined, with the youngest women having a prevalence of 8.5% (95% CI 5.7-12.5) and the oldest women 12.6% (95% CI 8.4-18.4). Women who had had basic education only, had the lowest prevalence of sub-RDT infection (8.3%, 95% CI 5.9-11.5) compared to women who had received no education, who had the highest prevalence (14.4%, 95% CI 9.6-21.1) during the first three study visits. A similar trend emerged when all the study visits were combined; women with basic education had a prevalence of 9.2% (95% CI 7.0-12.0) compared with women who had received no education who had a prevalence of 14.8% (95% CI 10.2-20.8). Secundigravid women had a higher prevalence of sub-RDT infection compared to primigravid women. The prevalence for secundigravid women was 13.5% (95% CI 10.0-17.9) during the first three visits compared to 7.0% (95% CI 4.8-10.3) for primigravid women; the same

observation was made when all the visits were combined with secundigravid women having a sub-RDT infection prevalence of 13.4% (95% CI 10.3-17.3) and primigravid women a prevalence of 8.2% (95% CI 6.0-11.2). Women enrolled during the late wet season had a prevalence of sub-RDT infection of 12.7% (95% CI 8.1-19.3) while women enrolled during the late dry season had a prevalence of 8.4% (95% CI 5.1-13.8) for the first three visits; when all the visits were combined the same pattern emerged with the highest prevalence being among women enrolled in the late wet season (11.9%, 95% CI 7.7-17.9) compared to 9.1% (95% CI 6.0-13.6) for women enrolled in the late dry season. There was no clear pattern in the risk of sub-RDT infection when the women are examined by their socio-economic status.

Table 28: Prevalence of sub-RDT malaria infections by background characteristics of women in IST arm

Characteristic	First three visits (v0-v2)		All visits (v0-v4)	
	n (%)	**95%CI**	**n (%)**	**95% CI**
Age group (years)	(N=634)		(N=819)	
<20	15(7.9)	4.8-12.6	23(8.5)	5.7-12.5
20-24	33(10.8)	7.8-14.8	41(11.0)	8.2-14.6
25+	15(11.0)	6.7-17.4	22(12.6)	8.4-18.4
Educational status	(N=634)		(N=819)	
None	21(14.4)	9.6-21.1	26(14.8)	10.2-20.8
Basic	32(8.3)	5.9-11.5	48(9.2)	7.0-12.0
Secondary	10(9.8)	5.4-17.5	12(9.8)	5.7-16.6
Gravidity	(N=631)		(N=816)	
1	24(7.0)	4.8-10.3	37(8.2)	6.0-11.2
2	39(13.5)	10.0-17.9	49(13.4)	10.3-17.3
Season of enrolment	(N=634)		(N=819)	
Early wet	19(9.0)	5.8-13.7	30(10.9)	7.7-15.1
Late wet	18(12.7)	8.1-19.3	19(11.9)	7.7-17.9
Early dry	12(10.5)	6.1-17.7	16(10.5)	6.5-16.5
Late dry	14(8.4)	5.1-13.8	21(9.1)	6.0-13.6
Socio-economic status	(N=633)		(N=817)	
Wealthiest	7(8.1)	3.9-16.2	12(11.4)	6.6-19.1
Wealthy	7(10.0)	4.8-19.6	10(10.9)	5.9-19.1
Medium	10(7.6)	4.1-13.7	16(9.0)	5.6-14.2
Poor	28(12.7)	8.9-17.8	34(11.9)	8.6-16.3
Poorest	11(8.8)	4.9-15.2	14(8.9)	5.3-14.5

5.4.2 Risk factors associated with sub-RDT malaria infection in pregnant women

Table 29 shows the results of logistic regression analyses of the risk of sub-RDT infections detected at the first three visits and all the visits combined.

Secundigravid women were twice as likely as primigravid women to have sub-RDT infections (OR 2.00, 95% CI 1.05-3.84, P=0.036) when the first three visits only were considered. When all the visits were combined the association between gravidity and sub-RDT infections was lost.

Table 29: Risk factors associated with sub-RDT malaria infections among pregnant women in Ghana

Characteristic	First three visits (v0-v2)						All visits (v0-v4)					
	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Age group(years)												
<20 years	1.00			1.00			1.00			1.00		
20-24 years	1.42	0.75-2.69	0.284	0.96	0.46-2.01	0.916	1.33	0.78-2.28	0.295	1.07	0.59-1.94	0.829
25+ years	1.44	0.68-3.06	0.340	0.88	0.34-2.25	0.785	1.55	0.84-2.88	0.164	1.10	0.52-2.34	0.799
Educational status												
None	1.00			1.00			1.00			1.00		
Basic	0.54	0.30-0.96	0.038	0.66	0.35-1.24	0.193	0.59	0.35-0.98	0.040	0.66	0.38-1.14	0.140
Secondary	0.65	0.29-1.46	0.298	0.96	0.38-2.41	0.926	0.63	0.30-1.30	0.212	0.65	0.29-1.50	0.317
Gravidity												
1	1.00			1.00			1.00			1.00		
2	2.05	1.20-3.50	0.008	2.00	1.05-3.84	0.036	1.73	1.10-2.71	0.018	1.54	0.90-2.63	0.116
Season of enrolment												
Early wet	1.00			1.00			1.00			1.00		
Late wet	1.47	0.74-2.92	0.265	1.48	0.74-2.97	0.266	1.10	0.60-2.04	0.749	1.10	0.59-2.04	0.765
Early dry	1.20	0.56-2.56	0.647	1.07	0.49-2.32	0.865	0.96	0.51-1.83	0.913	0.86	0.45-1.66	0.657
Late dry	0.94	0.45-1.93	0.857	0.90	0.43-1.88	0.787	0.82	0.46-1.48	0.508	0.80	0.44-1.44	0.456
Socio-economic Status												
Wealthiest	1.00			1.00			1.00			1.00		
Wealthy	1.25	0.42-3.76	0.686	1.47	0.48-4.53	0.505	0.95	0.39-2.30	0.901	1.03	0.41-2.58	0.956
Medium	0.93	0.34-2.55	0.892	1.04	0.36-2.96	0.945	0.77	0.35-1.69	0.508	0.76	0.33-1.76	0.526
Poor	1.64	0.69-3.90	0.266	1.80	0.70-4.66	0.226	1.05	0.52-2.11	0.892	1.02	0.47-2.23	0.946
Poorest	1.09	0.40-2.93	0.866	1.08	0.37-3.18	0.888	0.76	0.34-1.71	0.506	0.68	0.28-1.66	0.395

5.4.3 Predictors of sub-microscopic infections

Table 30 presents the prevalence of sub-microscopic infections by various background characteristics, estimated for the first three visits combined.

The prevalence for the first three visits was 19.9% (95% CI 16.9-23.4) and 24.1% (95% CI 20.8-27.8) for all five visits combined. The highest risk of sub-microscopic malaria infection was in the age group 20-24 years. Women with no formal education had the highest risk of sub-microscopic malaria infection in comparisons between socio-economic groups. During the first three visits the prevalence in women in this group was 20.7% (95% CI 16.2-26.1) increasing to 26.1% (95% CI 21.1-31.7) when all the visits were combined. Women in the IPTp-SP arm of the study had a lower risk of sub-microscopic infection compared to women in the IST arm. During the first three visits the risk of sub-microscopic infection was 16.7% (95% CI 14.0-19.8) for women in the IPTp-SP group compared to 18.4% (95% CI 15.6-21.6) for women in the IST group. A similar pattern emerged when all the visits were combined, with women in the IPTp-SP group having a prevalence of 20.9% (95% CI 17.9-24.2) compared to 22.8% (95% CI 19.7-26.3) for women in the IST group but for both of these sets of observations confidence limits substantially overlapped. Secundigravid women also had a greater risk of sub-microscopic infection compared to primigravid women. Secundigravid women had a prevalence of 20.0% (95% CI 16.9-23.6) during the first three visits while primigravid women had a prevalence of 15.5% (95% CI 13.0-18.4); for all the visits combined, secundigravid women had a prevalence of 24.2% (95% CI 20.9-27.9) while primigravid women had a prevalence of 19.9% (95 % CI 17.1-23.0). The risk of sub-microscopic infection was greatest among the women who were enrolled into the study in the late wet season followed by those enrolled early in the wet season. Women enrolled in the late wet season had a prevalence of sub-microscopic infection of 21.9% (95% CI 17.4-27.1) during the first three visits and 26.5% (95% CI 21.7-32.0) for all the visits combined while those enrolled early in

the wet season had a prevalence of 17.3% (95% CI 13.9-21.2) during the first three visits and 24.0% (95% CI 20.2-28.3) for all the visits combined. No clear pattern for the prevalence of sub-microscopic infection was seen when women were categorised by socio-economic status. It must be noted that even though some trends were observed as described above, the confidence intervals overlapped for nearly all the comparisons done.

Table 30: Risk of sub-microscopic malaria infections by background characteristics of study participants

Characteristic	First three visits (v0-v2)		All visits (v0-v4)	
	n (%)	95%CI	n (%)	95%CI
Age group (years)	(N=1,265)		(N=1,273)	
<20	65(15.4)	12.3-19.2	82(19.3)	15.9-23.4
20-24	115(19.9)	16.9-23.4	140(24.1)	20.8-27.8
25+	42(15.8)	11.9-20.7	56(20.8)	16.4-26.1
Educational status	(N=1,265)		(N=1,273)	
None	54(20.7)	16.2-26.1	68(26.1)	21.1-31.7
Basic	138(17.0)	14.5-19.7	168(20.5)	17.8-23.4
Secondary	30(15.7)	11.2-21.6	42(22.0)	16.7-28.4
Intervention Arm	(N=1,265)		(N=1,273)	
IPT	107(16.7)	14.0-19.8	134(20.9)	17.9-24.2
IST	115(18.4)	15.6-21.6	144(22.8)	19.7-26.3
Gravidity	(N=1,262)		(N=1,270)	
1	108(15.5)	13.0-18.4	140(19.9)	17.1-23.0
2	113(20.0)	16.9-23.6	137(24.2)	20.9-27.9
Season of enrolment	(N=1,265)		(N=1,273)	
Early wet	73(17.3)	13.9-21.2	102(24.0)	20.2-28.3
Late wet	61(21.9)	17.4-27.1	74(26.5)	21.7-32.0
Early dry	34(15.2)	11.0-20.5	40(17.8)	13.3-23.4
Late dry	54(15.9	12.4-20.2	62(18.1)	14.3-22.5
Socio-economic status	(N=1,262)		(N=1,269)	
Wealthiest	25(15.7)	10.8-22.3	33(20.6)	15.0-27.6
Wealthy	21(13.1)	8.7-19.3	28(17.5)	12.4-24.2
Medium	43(15.6)	11.8-20.4	57(20.7)	16.3-25.9
Poor	89(20.4)	16.9-24.5	103(23.5)	19.7-27.7
Poorest	44(19.0)	14.4-24.6	57(24.4)	19.3-30.3

5.4.4 Risk factors associated with sub-microscopic malaria infection in pregnant women

Table 31 shows the results of logistic regression analysis modelling of the effects of various background characteristics on sub-microscopic malaria infections among women in the study. The analysis was done separately for the first three study visits when interventions were given and also for all the visits including delivery. For the first three study visits, none of the risk factors examined was significantly associated with sub-microscopic infections. In univariate logistic regression, gravidity appeared to be related to sub-microscopic infection but this effect was attenuated in multivariate analysis.

When all the study visits were combined, women who were enrolled in the late dry season were less likely to have sub-microscopic infection compared to women enrolled in the early wet season (OR 0.70, 95% CI 0.49-0.99, P=0.047) but as a large number of comparisons were made this may be a chance finding.

Table 31: Risk factors associated with sub-microscopic malaria infections among pregnant women in Ghana

Characteristic	First three visits (v0-v2)						All visits (v0-v4)					
	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Age Group												
<20 years	1.00			1.00			1.00			1.00		
20-24 years	1.37	0.98-1.91	0.067	1.23	0.85-1.79	0.271	1.33	0.98-1.80	0.071	1.22	0.86-1.71	0.265
25+ years	1.03	0.67-1.57	0.892	0.90	0.54-1.50	0.686	1.10	0.75-1.60	0.635	0.95	0.60-1.50	0.820
Educational status												
None	1.00			1.00			1.00			1.00		
Basic	0.78	0.55-1.11	0.174	0.88	0.61-1.28	0.515	0.73	0.53-1.01	0.057	0.81	0.58-1.15	0.241
Secondary	0.71	0.43-1.17	0.180	0.88	0.50-1.53	0.641	0.80	0.52-1.24	0.320	0.94	0.57-1.55	0.813
Gravidity												
1	1.00			1.00			1.00			1.00		
2	1.37	1.02-1.83	0.034	1.33	0.95-1.87	0.100	1.29	0.99-1.68	0.064	1.24	0.91-1.69	0.181
Intervention arm												
IPT	1.00			1.00			1.00			1.00		
IST	1.12	0.84-1.50	0.432	1.07	0.80-1.44	0.641	1.12	0.86-1.46	0.400	1.08	0.82-1.42	0.577
Season of enrolment												
Early wet	1.00			1.00			1.00			1.00		
Late wet	1.34	0.92-1.96	0.129	1.38	0.94-2.03	0.097	1.14	0.81-1.62	0.450	1.17	0.82-1.66	0.378
Early dry	0.86	0.55-1.34	0.499	0.84	0.54-1.32	0.461	0.68	0.46-1.03	0.069	0.68	0.45-1.02	0.063
Late dry	0.91	0.62-1.34	0.625	0.90	0.61-1.33	0.612	0.70	0.49-0.99	0.045	0.70	0.49-0.99	0.047
Socio-economic Status												
Wealthiest	1.00			1.00			1.00			1.00		
Wealthy	0.91	0.50-1.64	0.743	0.79	0.42-1.51	0.483	0.82	0.47-1.43	0.477	0.82	0.46-1.46	0.496
Medium	1.06	0.63-1.77	0.838	0.94	0.54-1.66	0.839	1.00	0.62-1.62	0.995	0.99	0.59-1.64	0.960
Poor	1.34	0.84-2.16	0.221	1.29	0.76-2.20	0.342	1.18	0.76-1.84	0.464	1.17	0.72-1.90	0.529
Poorest	1.28	0.76-2.15	0.361	1.17	0.65-2.11	0.597	1.24	0.76-2.01	0.386	1.22	0.72-2.08	0.465

5.5 Effects of sub-patent malaria infections

This section presents the results of analyses of the effects of sub-patent malaria infection on three outcomes of interest: birth weight/low birth weight, placental malaria and blood haemoglobin/anaemia. The results are presented for both sub-microscopic infections and sub-RDT infections for the first three visits and then for all the visits combined.

5.5.1 Risk factors of low birth weight

Table 32 shows the incidence of low birth weight among women in the study by various background characteristics as well as by their malaria infection status determined by microscopy and PCR on one hand and RDT and PCR on the another hand. Primigravid women had a higher incidence of low birth weight babies (19.3%, 95% CI 16.3-22.7) compared to secundigravid women (13.9%, 95% CI 11.1-17.3). Women enrolled during the late dry season had the highest incidence of low birth weight (23.6%, 95% CI 18.9-29.1) while the women enrolled during the late wet season had the lowest prevalence (13.2%, 95% CI 9.5-18.1). Malaria infection status as determined by RDT appeared to have an inverse association with the incidence of low birth weight. Women who were uninfected had a higher incidence of low birth weight compared to women who had RDT positive malaria or even sub-RDT infections considering the first three visits alone and all the visits combined.

Table 32: Incidence of low birth weight by background characteristics of study participants

Characteristic	n	Incidence (%)	95%CI	p-value
Age group (years)	(N=1,078)			0.122
<20	73	19.8	16.0-24.2	
20-24	79	16.5	13.5-20.1	
25+	31	13.4	9.6-18.5	
Educational status	(N=1,078)			0.151
None	27	12.6	8.7-17.7	
Basic	126	18.2	15.5-21.3	
Secondary	30	17.4	12.5-23.9	
Intervention Arm	(N=1,078)			0.632
IPT	86	16.4	13.5-19.8	
IST	97	17.5	14.6-20.9	
Gravidity	(N=1,076)			0.019
1	116	19.3	16.3-22.7	
2	66	13.9	11.1-17.3	
Season of enrolment	(N=1,078)			0.006
Early wet	61	16.1	12.8-20.2	
Late wet	32	13.2	9.5-18.1	
Early dry	26	14.0	9.7-19.8	
Late dry	64	23.6	18.9-29.1	
Socio-economic status	(N=1,075)			0.662
Wealthiest	27	19.3	13.6-26.7	
Wealthy	20	14.1	9.3-20.9	
Medium	42	18.8	14.2-24.4	
Poor	64	17.3	13.8-21.5	
Poorest	30	15.1	10.7-20.8	
Microscopy status v0-v2	(N=1,047)			0.368
Uninfected	58	18.9	14.9-23.7	
Microscopic	83	15.2	12.4-18.4	
Sub-microscopic	33	17.1	12.4-23.1	
RDT status v0-v2	(N=532)			0.034
Uninfected	29	25.4	18.2-34.3	
RDT positive	57	15.8	12.4-19.9	
Sub-RDT	7	12.3	5.9-23.8	
Microscopy statusv0-v4	(N=1,049)			0.376
Uninfected	48	19.4	14.9-24.8	
Microscopic	86	15.4	12.6-18.6	
Sub-microscopic	40	16.5	12.4-21.8	
RDT status v0-v4	(N=539)			0.037
Uninfected	26	26.0	18.3-35.5	
RDT positive	58	16.0	12.6-20.1	
Sub-RDT	10	13.2	7.2-22.9	

5.5.2 Effect of sub-patent malaria infection on birth weight

Table 33 shows the results of linear regression analysis of mean birth weight and malaria infection status by RDT/PCR and microscopy/PCR. Adjusting for age group, education, gravidity, socio-economic status and season of enrolment, there was no significant association between mean birth weight and malaria infection status by RDT/PCR. Similarly, adjusting for the same factors, in addition to the intervention arm of the study women, there was no significant association between mean birth weight and malaria infection status by microscopy/PCR.

However, women who had microscopic malaria infection at delivery were 2.05 times more likely to have a low birth weight baby compared to women who had no malaria infection (95% CI 1.25-3.37, P=0.005) controlling for age group, education gravidity, socio-economic status and season of enrolment (Table 34). There was no significant association between sub-RDT malaria infection at any time or period in pregnancy and low birth weight.

Table 33: Effect of malaria infection status on birth weight among pregnant women in Ghana

Characteristic	Mean birth weight(kg)	95% CI	Crude coefficient	95% CI	P-value	Adjusted coefficient	95% CI	P-value	Covariates adjusted for
Microscopy status v0-v2									Age group, Education, Socio-economic status, Gravidity, Season of enrolment Intervention arm
Uninfected	2.80	2.74-2.84	0.00			0.00			
Microscopic	2.77	2.73-2.80	-0.02	-0.08-0.04	0.444	-0.002	-0.06-0.06	0.949	
Sub-microscopic	2.77	2.71-2.84	-0.01	-0.09-0.06	0.712	-0.009	-0.09-0.7	0.819	
Microscopy status v0-v4									Age group, Education, Socio-economic status, Gravidity, Season of enrolment, Intervention arm
Uninfected	2.79	2.73-2.85	0.00			0.00			
Microscopic	2.77	2.73-2.80	-0.02	-0.09-0.04	0.445	0.004	-0.06-0.07	0.903	
Sub-microscopic	2.77	2.72-2.83	-0.02	-0.10-0.06	0.605	-0.009	-0.09-0.07	0.820	
Microscopy status at deliver (v4)									Age group, Education, Socio-economic status, Gravidity, Season of enrolment Intervention arm
Uninfected	2.79	2.75-2.82	0.00			0.00			
Microscopic	2.70	2.61-2.79	-0.08	-0.17-0.01	0.070	-0.04	-0.13-0.05	0.382	
Sub-microscopic	2.69	2.55-2.84	-0.09	-0.23-0.05	0.195	-0.10	-0.25-0.04	0.146	
RDT status v0-v2									Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	2.73	2.64-2.82	0.00			0.00			
RDT positive	2.76	2.71-2.80	0.03	-0.06-0.12	0.515	0.06	-0.04-0.15	0.251	
Sub-RDT	2.84	2.74-2.94	0.11	-0.03-0.25	0.115	0.10	-0.04-0.24	0.179	
RDT status v0-v4									Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	2.71	2.61-2.82	0.00			0.00			
RDT positive	2.76	2.72-2.81	0.05	-0.05-0.15	0.329	0.08	-0.02-0.19	0.118	
Sub-RDT	2.83	2.73-2.93	0.12	-0.02-0.25	0.090	0.11	-0.02-0.25	0.099	
RDT status at delivery (v4)									Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	2.79	2.74-2.85	0.00			0.00			
RDT positive	2.75	2.65-2.86	-0.04	-0.16-0.07	0.467	-0.02	-0.14-0.10	0.767	
Sub-RDT	2.79	2.48-3.10	0.00	-0.20-0.20	0.999	-0.01	-0.22-0.20	0.934	

Table 34: Effect of malaria infection status on low birth weight among pregnant women in Ghana

Characteristic	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Covariates adjusted for
Microscopy status v0-v2							Age group, Education, Gravidity, Socio-economic status, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	0.77	0.53-1.11	0.161	0.78	0.53-1.16	0.225	
Sub-microscopic	0.89	0.55-1.42	0.613	0.92	0.56-1.51	0.752	
Microscopy status v0-v4							Age group, Education, Gravidity, Socio-economic status, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	0.76	0.51-1.12	0.163	0.74	0.49-1.11	0.145	
Sub-microscopic	0.83	0.52-1.31	0.416	0.85	0.52-1.37	0.504	
Microscopy status at delivery (v4)							Age group, Education, Gravidity, Socio-economic status, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	2.36	1.47-3.78	<0.001	2.05	1.25-3.37	0.005	
Sub-microscopic	1.45	0.65-3.26	0.362	1.57	0.69-3.57	0.278	
RDT status v0-v2							Age group, Education, Gravidity, Socio-economic status, Season of enrolment
Uninfected	1.00			1.00			
RDT Positive	0.55	0.33-0.91	0.021	0.58	0.33-1.01	0.056	
Sub-RDT	0.41	0.17-1.01	0.051	0.50	0.20-1.26	0.143	
RDT status v0-v4							Age group, Education, Gravidity, Socio-economic status, Season of enrolment
Uninfected	1.00			1.00			
RDT Positive	0.54	0.32-0.92	0.023	0.57	0.32-1.01	0.055	
Sub-RDT	0.43	0.19-0.96	0.040	0.49	0.21-1.12	0.090	
RDT status at delivery (v4)							Age group, Education, Gravidity, Socio-economic status, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	1.55	0.77-3.12	0.217	1.63	0.76-3.47	0.208	
Sub-RDT	1.18	0.32-4.29	0.807	1.31	0.34-5.04	0.698	

5.5.3 Active placental malaria prevalence by background characteristics of study participants

Table 35 shows the prevalence of active placental malaria by background characteristics of the study participants. The season of enrolment of the women was significantly associated with active placental malaria (P<0.001). Women who had microscopic or sub-microscopic peripheral parasitaemia at any one of the 4 antenatal visits also had higher prevalence of active placental malaria compared to women who did not have peripheral parasitaemia in all four ANC visits (P=0.028).

Table 35: Prevalence of active placental malaria by background characteristics of study participants

Characteristic	n	Prevalence (%)	95%CI	p-value
Age group (years)	(N=786)			0.085
<20	91	34.1	28.6-40.0	
20-24	97	27.6	23.2-32.6	
25+	42	25.0	19.0-32.1	
Educational status	(N=786)			0.248
None	34	23.8	17.5-31.5	
Basic	155	30.0	26.2-34.2	
Secondary	41	32.3	24.7-40.9	
Intervention Arm	(N=786)			0.783
IPT	115	29.7	25.4-34.5	
IST	115	28.8	24.6-33.5	
Gravidity	(N=785)			0.105
1	141	31.6	27.5-36.1	
2	89	26.3	21.8-31.2	
Season of enrolment	(N=786)			<0.001
Early wet	112	42.1	36.3-48.2	
Late wet	27	15.8	11.0-22.1	
Early dry	29	19.9	14.1-27.2	
Late dry	62	30.5	24.6-37.2	
Socio-economic status	(N=785)			0.804
Wealthiest	31	30.1	22.0-39.7	
Wealthy	39	33.1	25.1-42.1	
Medium	47	27.8	21.6-35.1	
Poor	80	29.7	24.6-35.5	
Poorest	33	26.2	19.2-34.6	
Microscopy status v0-v2	(N=764)			0.690
Uninfected	65	28.0	22.6-34.2	
Microscopic	110	28.0	23.8-32.6	
Sub-microscopic	44	31.7	24.4-39.9	
RDT status v0-v2	(N=386)			0.817
Uninfected	25	29.8	20.9-40.5	
RDT positive	72	27.6	22.5-33.4	

Sub-RDT	10	24.4	13.5-40.0	
Microscopy status v0-v4	(N=765)			0.028
Uninfected	40	22.2	16.7-28.9	
Microscopic	117	28.7	24.5-33.3	
Sub-microscopic	62	35.0	28.3-33.3	
RDT status v0-v4	(N=392)			0.421
Uninfected	22	31.0	21.3-42.7	
RDT positive	71	26.9	21.9-32.6	
Sub-RDT	20	35.1	23.8-48.4	

5.5.4 Effect of sub-patent malaria on active placental malaria prevalence

Table 36 shows the association between peripheral malaria infection status detected at ANC visits and presence of active placental malaria adjusted for the effects of age group, educational status, gravidity, intervention arm, season of enrolment and socio-economic status. Women with sub-microscopic malaria infections detected at any one of the 4 visits combined were 2.01 times more likely to have active placental malaria compared to women who were uninfected (95% CI 1.23-3.30, P=0.006) but women with microscopic infections were only 1.35 times more likely to have active placental infection and this was not statistically significant.

Adjusting for the same factors, women who had sub-microscopic malaria infection at the time of delivery were 3.11 times more likely to have active placental malaria infection (95% CI 1.37-7.06, P=0.007) while women who had microscopic malaria infection at delivery were 6.21 times more likely to have active placental malaria (95% CI 3.75-10.30, P<0.001).

RDT infection status for the first three visits and all the visits combined was not associated with active placental malaria. However, adjusting for age group, education, gravidity, season of enrolment and socio-economic status, women who had a positive RDT at delivery were 2.04 times more likely to have active placental malaria (95% CI 1.06-3.90, P=0.032) while women who had a sub-RDT malaria infection were 5.23 times more likely to have active placental malaria (95% CI 1.68-16.29, P=0.004).

Table 36: Effect of malaria infection status on active placental malaria among pregnant women in Ghana

Characteristic	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Covariates adjusted for
Microscopy status v0-v2							Age group, Education, Socio-economic status Gravidity, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	1.00	0.70-1.43	0.994	1.00	0.68-1.47	0.999	
Sub-microscopic	1.19	0.75-1.88	0.457	1.34	0.82-2.18	0.239	
Microscopy status v0-v4							Age group, Education, Socio-economic status Gravidity, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	1.41	0.93-2.12	0.104	1.35	0.87-2.08	0.180	
Sub-microscopic	1.89	1.18-3.01	0.008	2.01	1.23-3.30	0.006	
Microscopy status at delivery (v4)							Age group, Education, Socio-economic status Gravidity, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	6.26	3.93-9.96	<0.001	6.21	3.75-10.30	<0.001	
Sub-microscopic	3.61	1.67-7.84	0.001	3.11	1.37-7.06	0.007	
RDT status v0-v2							Age group, Education, Socio-economic status Gravidity, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	0.90	0.52-1.54	0.700	0.86	0.48-1.55	0.626	
Sub-RDT	0.76	0.32-1.79	0.531	0.83	0.34-2.06	0.693	
RDT status v0-v4							Age group, Education, Socio-economic status Gravidity, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	0.82	0.46-1.45	0.495	0.79	0.43-1.47	0.463	
Sub-RDT	1.20	0.57-2.53	0.623	1.29	0.58-2.87	0.529	
RDT status at delivery (v4)							Age group, Education, Socio-economic status Gravidity, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	2.25	1.23-4.13	0.008	2.04	1.06-3.90	0.032	
Sub-RDT	4.59	1.59-13.30	0.005	5.23	1.68-16.29	0.004	

5.5.5 Effects of sub-patent malaria infection on the prevalence of anaemia

The prevalence of anaemia in pregnancy (haemoglobin< 11grams/dl) among the women in the study was significantly associated with age group (P=0.018), gravidity (P=0.042) and season of enrolment (P=0.001) [Table 37]. Younger and primigravid women had a higher prevalence of anaemia compared to older and secundigravid women. There was no significant difference in prevalence of anaemia by study intervention arm.

Table 37: Prevalence of anaemia by background characteristics of study participants

Characteristic	n	Prevalence (%)	95%CI	p-value
Age group (years)	(N=528)			0.018
<20	93	58.1	50.3-65.6	
20-24	130	57.0	50.5-63.3	
25+	61	43.6	35.6-51.9	
Educational status	(N=528)			0.783
None	56	54.9	45.1-64.3	
Basic	181	54.4	49.0-59.7	
Secondary	47	50.5	40.4-60.6	
Intervention Arm	(N=528)			0.638
IPT	135	52.7	46.6-58.8	
IST	149	54.8	48.8-60.6	
Gravidity	(N=526)			0.042
1	158	57.9	51.9-63.6	
2	124	49.0	42.9-55.2	
Season of enrolment	(N=528)			0.001
Early wet	104	54.7	47.6-61.7	
Late wet	60	47.6	39.0-56.4	
Early dry	44	43.6	34.2-53.4	
Late dry	76	68.5	59.2-76.5	
Socio-economic status	(N=528)			0.075
Wealthiest	32	39.5	29.4-50.6	
Wealthy	42	56.0	44.6-66.9	
Medium	71	58.2	49.2-66.7	
Poor	95	54.3	46.8-61.6	
Poorest	44	58.7	47.2-69.3	
Microscopy status v0-v2	(N=515)			0.426
Uninfected	76	49.0	41.2-56.9	
Microscopic	150	55.2	49.2-61.0	
Sub-microscopic	49	55.7	45.1-65.8	
RDT status v0-v2	(N=262)			0.703
Uninfected	25	49.0	35.5-62.7	
RDT positive	100	55.3	47.9-62.4	
Sub-RDT	17	56.7	38.5-73.2	
Microscopy status v0-v3	(N=515)			0.249
Uninfected	65	47.45	39.2-55.9	
Microscopic	151	56.13	50.1-62.0	

Sub-microscopic	59	54.13	44.7-63.3	
RDT status v0-v3	(N=262)			0.708
Uninfected	25	49.0	35.5-62.7	
RDT positive	100	55.6	48.2-62.7	
Sub-RDT	17	54.8	337.1-71.4	

5.5.6 Effect of sub-patent malaria infection on anaemia in pregnancy

Table 38 shows the association between peripheral malaria infection status and maternal anaemia adjusted for the effect of age group, education, gravidity, intervention arm, socio-economic status, and season of enrolment. Women who had microscopic malaria infection at 38 weeks (visit 3) when anaemia was assessed, were 2.38 times more likely to have anaemia (95% CI 1.19-4.76, P=0.014) compared to women who were uninfected. Sub-patent malaria infections was not associated with maternal anaemia in this study population.

5.5.7 Effect of sub-patent malaria infection on blood haemoglobin concentration in pregnancy

Table 39 shows association between peripheral malaria infection status and mean blood haemoglobin concentration measured at 38 weeks of gestation (visit 3). Adjusting for age group, educational status, gravidity, intervention arm, socio-economic status and season of enrolment, the mean haemoglobin level of women who had microscopic malaria infection at 38 weeks gestation was on the average 0.54grams/dl less [95% CI -0.91-(-)0.18, P=0.004] than that of women who had no malaria infection. There was no significant relationship between sub-patent malaria infections and blood haemoglobin in pregnancy.

Table 38: Effect of malaria infection status on anaemia in pregnancy among pregnant women in Ghana

Characteristic	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Covariates adjusted for
Microscopy status v0-v2							Age group, Education, Socio-economic status, Gravidity, Season of enrolment Intervention arm
Uninfected	1.00			1.00			
Microscopic	1.28	0.86-1.90	0.224	1.33	0.86-2.06	0.200	
Sub-microscopic	1.31	0.77-2.21	0.319	1.38	0.78-2.42	0.264	
Microscopy status v0-v3							Age group, Education, Socio-economic status, Gravidity, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	1.42	0.94-2.14	0.098	1.38	0.88-2.17	0.158	
Sub-microscopic	1.31	0.79-2.16	0.298	1.28	0.74-2.19	0.375	
Microscopy status at 38 weeks (v3)							Age group, Education, Socio-economic status, Gravidity, Season of enrolment, Intervention arm
Uninfected	1.00			1.00			
Microscopic	3.06	1.59-5.90	0.001	2.38	1.19-4.76	0.014	
Sub-microscopic	1.00	0.44-2.27	0.991	0.90	0.38-2.13	0.818	
RDT status v0-v2							Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	1.28	0.69-2.39	0.431	1.36	0.66-2.81	0.408	
Sub-RDT	1.36	0.55-3.37	0.506	1.49	0.54-4.09	0.442	
RDT status v0-v3							Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	1.00			1.00			
RDT positive	1.30	0.70-2.42	0.409	1.39	0.67-2.89	0.371	
Sub-RDT	1.26	0.52-3.09	0.609	1.32	0.49-3.57	0.588	

Table 39: Effect of malaria infection status on blood haemoglobin among pregnant women in Ghana

Characteristic	Mean Hb g/dl	95% CI	Crude coefficient	95% CI	P-value	Adjusted coefficient	95% CI	P-value	Covariates adjusted for
Microscopy status v0-v2									Age group, Education, Socio-economic status, Gravidity, Season of enrolment Intervention arm
Uninfected	10.9	10.7-11.1	0.00			0.00			
Microscopic	10.8	10.6-10.9	-0.13	-0.38-0.12	0.298	-0.13	-0.39-0.13	0.316	
Sub-microscopic	10.8	10.6-11.1	-0.10	-0.43-0.24	0.567	-0.09	-0.42-0.24	0.602	
Microscopy status v0-v3									Age group, Education, Socio-economic status, Gravidity, Season of enrolment, Intervention arm
Uninfected	11.0	10.8-11.2	0.00			0.00			
Microscopic	10.8	10.6-10.9	-0.24	-0.50-0.02	0.075	-0.20	-0.46-0.07	0.148	
Sub-microscopic	10.8	10.6-11.1	-0.18	-0.50-0.14	0.276	-0.12	-0.44-0.20	0.454	
Microscopy status at 38 weeks (v3)									Age group, Education, Socio-economic status, Gravidity, Season of enrolment, Intervention arm
Uninfected	10.9	10.8-11.1	0.00			0.00			
Microscopic	10.2	9.9-10.5	-0.72	-1.08-(-)0.37	<0.001	-0.54	-0.91-(-)0.18	0.004	
Sub-microscopic	10.9	10.2-11.6	-0.09	-0.59-0.42	0.741	0.00	-0.50-0.51	0.993	
RDT status v0-v2									Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	10.9	10.5-11.2	0.00			0.00			
RDT positive	10.7	10.5-10.9	-0.13	-0.53-0.28	0.535	-0.19	-0.60-0.22	0.364	
Sub-RDT	10.8	10.3-11.2	-0.11	-0.70-0.47	0.703	-0.16	-0.72-0.40	0.577	
RDT status v0-v3									Age group, Education, Socio-economic status, Gravidity, Season of enrolment
Uninfected	10.9	10.5-11.2	0.00			0.00			
RDT positive	10.7	10.5-10.9	-0.13	-0.54-0.27	0.517	-0.20	-0.61-0.21	0.333	
Sub-RDT	10.8	10.3-11.3	-0.08	-0.66-0.50	0.787	-0.11	-0.66-0.44	0.700	

5.6 Summary

The prevalence of sub-microscopic malaria declined from 10.6% at the first ANC visit to 5.1% at delivery as the prevalence of malaria declined. The risk of sub-microscopic malaria infection for the first three scheduled ANC visits combined was 17.6% and it was 21.8% when all four ANC visits were combined. The highest prevalence of sub-RDT infection was observed at delivery (6.3%). The risk of sub-RDT infection was 10.1% for the first three ANC visits and 13.5% for all four ANC visits combined.

The season of enrolment was the only predictor of sub-microscopic infection. Gravidity predicted sub-RDT infection when only the first three visits were considered.

There was no significant association between malaria infection status during pregnancy and mean birth weight of babies. There was no significant association between sub-patent malaria infection and low birth weight. Microscopic peripheral malaria parasitaemia was however, associated with low birth weight. Women with microscopic or sub-microscopic malaria infections at delivery were more likely to have active placental malaria. Also, women who had RDT positive malaria at delivery were 2.04 times more likely to have active placental malaria while women who had sub-RDT malaria at delivery were 5.23 times more likely to have active placental malaria.

Women who had microscopic malaria infections at 38 weeks of gestation were more likely to have anaemia compared to women who were uninfected. There was no significant association between sub-patent malaria and anaemia in pregnancy.

CHAPTER SIX

Prevalence of Falciparum and Non-falciparum Malaria Infection in Pregnancy in Four West African Countries

6.1 Introduction

This chapter presents the findings from an analysis of the prevalence of falciparum and non-falciparum malaria infections in a sub-set of pregnant women who were enrolled into a clinical trial of IPTp-SP versus IST conducted in Burkina Faso, the Gambia, Ghana and Mali. This subset comprises women who were tested for malaria on their day of recruitment into the study. An analysis of the prevalence of non-falciparum malaria as detected by PCR is first presented for all the four countries combined. Analysis of the non-falciparum infections missed by RDT testing is then presented. Blood smear microscopy detected very few non-falciparum infections in this study. Each of the two microscopists detected only six (6) cases of non-falciparum malaria compared to forty (40) cases detected by PCR. Out of the cases detected by the two microscopists, only one case of *P. malariae* mono infection was detected by both readers independently and therefore confirmed. The prevalence of non-falciparum malaria was too low to allow meaningful country-level analyses. The analyses of non-falciparum malaria is followed by presentation of findings from analyses of malaria infection (all species) detected by microscopy and PCR.

6.2 Prevalence of falciparum and non-falciparum malaria as detected by PCR

Table 40 shows the prevalence of Plasmodium species (mono and mixed) by country as detected by PCR. A total of 39.01% of the women had malaria infection, a great majority of them being *P. falciparum* mono infections (38.39%), with the remainder being largely mixed

infections of *P. falciparum* with other species and a few non-falciparum mono infections. Only four cases of *P. malariae* mono infections were found among the 2,347 women, giving a prevalence of 0.17%. There were 5 cases of *P. ovale* mono infections among the women in this sub-sample, giving a prevalence of 0.22%; three of these were cases of *P. ovale curtisi* (0.13%) while two were cases of *P. ovale wallikeri* (0.09%). There were 2 cases of *P. vivax* mono infections accounting for a prevalence of 0.09%. These two cases of *P. vivax* mono infections were women enrolled at the Mali site of the trial. In total therefore, there were only 11 cases of non-falciparum mono infections among the 2,347 women. In contrast, there were 29 women who had mixed infections of falciparum and non-falciparum malaria infections. Thirteen women (0.55%) had mixed infections of *P. falciparum* and *P. malariae*, while 12 women (0.51%) had mixed infections of *P. falciparum* and *P. ovale* infections; there were five cases of *P. falciparum* mixed with *P. ovale curtisi* (0.21%) and seven cases of *P. falciparum* mixed with *P. ovale wallikeri* (0.30%). Two women (0.09%) had mixed infections of *P. falciparum* and *P. vivax*. These women were all enrolled at the Mali site as observed with the 2 cases of *P. vivax* mono infections presented earlier. There were also two cases of three-specie mixed infections of *P. falciparum*, *P. malariae* and *P. ovale curtisi*. One of these women was enrolled in Ghana and the other in Mali.

On country level the prevalence of *P. falciparum* infections ranged from 13.38% in the Gambia to 26.08% in Mali, to 58.86% in Ghana to 59.16% in Burkina Faso. Of the four cases of *P. malariae* mono infection, 3 were from Mali and one was from Burkina Faso. Of the five *P. ovale* mono infections, two were from women enrolled in the Gambia (comprising one each of *P. ovale curtisi* and *P. ovale wallikeri*) and one each from each of the other three countries in the trial. Ten of the thirteen mixed *P. falciparum* and *P. malariae* infections were obtained from women enrolled in Mali while the remaining three cases were from women enrolled in Ghana. Of the 12 mixed *P. falciparum* and *P. ovale* cases detected, 5 were obtained from

women enrolled in Ghana (two cases of *P. ovale curtisi* mixed with *P. falciparum* and three cases of *P. ovale wallikeri* mixed with *P. falciparum*) while 4 were from women enrolled in Mali (one *P. ovale curtisi* mixed with *P. falciparum* and three cases of *P. ovale wallikeri* mixed with *P. falciparum*) and 3 were from women from the Burkina Faso site. Apart from the 2 cases of *P. ovale* mono infections detected in women enrolled in the Gambia there were no other cases of non-falciparum malaria, mono or mixed detected among the women in that country. Mali had the highest incidence of non-falciparum malaria with 23 cases out of a total of 40 women who either had mono or mixed infections. Ghana was next with a total of 10 mono and mixed infections while Burkina Faso had 5 non-falciparum infections in all.

Table 40: Prevalence of Plasmodium species (mono and mixed) by country as detected by PCR

Species	Overall (%)	Burkina Faso n (%)	Gambia n (%)	Ghana n (%)	Mali n (%)
Plasmodium falciparum	901(38.39)	281(59.16)	97(13.38)	402(58.86)	121(26.08)
Plasmodium malariae	4(0.17)	1(0.21)	0(0.00)	0(0.00)	3(0.65)
Plasmodium ovale curtisi	3(0.13)	1(0.21)	1(0.14)	0(0.00)	1(0.22)
Plasmodium ovale wallikeri	2(0.09)	0(0.00)	1(0.14)	1(0.15)	0(0.00)
Plasmodium vivax	2(0.09)	0(0.00)	0(0.00)	0(0.00)	2(0.43)
Pf + Pm	13(0.55)	0(0.00)	0(0.00)	3(0.44)	10(2.16)
Pf + Poc	5(0.21)	2(0.42)	0(0.00)	2(0.29)	1(0.22)
Pf+ Pow	7(0.30)	1(0.21)	0(0.00)	3(0.44)	3(0.65)
Pf + Pv	2(0.09)	0(0.00)	0(0.00)	0(0.00)	2(0.43)
Pf + Pm + Poc	2(0.09)	0(0.00)	0(0.00)	1(0.15)	1(0.22)
Plasmodium negative	1,406(59.91)	189(39.79)	626(86.34)	271(39.68)	320(68.97)
Total	2,347(100.00)	475(100.00)	725(100.00)	683(100.00)	464(100.00)

Legend: *Pf: Plasmodium falciparum; Pm: Plasmodium malariae; Poc: Plasmodium ovale curtisi; Pow: Plasmodium ovale wallikeri; Pv: Plasmodium vivax*

6.3 Falciparum and non-falciparum malaria undetected by RDT

Table 41 show proportions of women who had PCR positive malaria which was detected (and undetected) by RDT. Out of a total of 740 women who had malaria infections detected by PCR, the RDT used was able to detect 86.9% of these infections leaving 13.1% undetected. The type of RDT used in this trial, the First Response® Combo HRP2/pLDH test can detect the presence of falciparum and non-falciparum species.

However, the test did not detect 12.6% of falciparum malaria detected by PCR.

Of 3 cases of *P. malariae* mono infections detected by PCR, the RDT detected 2 (66.6%) leaving 1 (33.3%) undetected. Out of 4 cases of *P. ovale* detected by PCR the RDT did not detect 3 cases (75.0%). Similarly, all the 2 cases of *P. vivax* detected by PCR were not detected by the RDT.

The picture is different for the mixed infections of falciparum and non-falciparum malaria. With the exception of two cases (18.2%) of *P. falciparum* and *P. malariae* mixed infections out of 11 cases which were detected by PCR but missed by RDT, all the remaining mixed infections, comprising 9 cases of *P. falciparum* mixed with *P. ovale*, 2 cases of *P. falciparum* mixed with *P. vivax*, and 2 cases of *P. falciparum* mixed with *P. malariae* and *P. ovale* were all detected by the RDT.

Table 41: PCR positive malaria detected by RDT

Species(PCR Results)	RDT Negative(%)	RDT Positive(%)
P. falciparum	89(12.6)	618(87.4)
P. malariae	1(33.3)	2(66.7)
P. ovale curtisi	1(50.0)	1(50.0)
P. ovale wallikeri	2(100.0)	0(0.0)
P. vivax	2(100.0)	0(0.0)
Pf + Pm	2(18.2)	9(81.8)
Pf + Poc	0(0.0)	3(100.0)
Pf+Pow	0(0.0)	6(100.0)
Pf + Pv	0(0.0)	2(100.0)
Pf + Pm + Poc	0(0.0)	2(100.0)
Total	97(13.1)	643(86.9)

6.4 Characteristics of pregnant women with a non-falciparum infection detected by PCR at first antenatal clinic attendance.

Because of the paucity of information on the epidemiology of non-falciparum malaria in pregnancy in sub-Saharan Africa a separate series of analyses were undertaken for women identified to have a non-falciparum malaria infection by PCR (40 out of 2,348 women). The background characteristics of these women are shown in Table 42. Study participants resident in Mali had the highest prevalence of non-falciparum malaria (5.0%; 95% CI 3.3-7.4) while study participants resident in the Gambia had the lowest prevalence (0.3%; 95% CI 0.1-1.1). The highest prevalence of non-falciparum malaria was obtained in the late wet season (3.0%; 95% CI 1.7-5.2). The highest prevalence was in the age group 25 years and over (2.0%; 95% CI 0.9-4.4). Women in their first pregnancy had a slightly higher prevalence of non-falciparum malaria (1.8%; 95% CI 1.2-2.7) than women in their second pregnancy (1.6; 95% CI 1.0-2.6). There was no clear association between the prevalence of non-falciparum malaria and educational status and wealth index

Table 42: Characteristics of women with a non-falciparum malaria infection detected at first antenatal clinic attendance by PCR

Parameter	n	Prevalence (%)	95%CI	p-value
Country	N=2,348			<0.001
Burkina Faso	5	1.1	0.4-2.5	
Gambia	2	0.3	0.1-1.1	
Ghana	10	1.5	0.8-2.7	
Mali	23	5.0	3.3-7.4	
Season of enrolment	N=2,348			0.102
Early wet	8	1.0	0.5-2.0	
Late wet	12	3.0	1.7-5.2	
Early dry	8	1.5	0.8-3.0	
Late dry	12	1.88	1.1-3.3	
Age group (years)	N=2,348			0.657
<20	20	1.9	1.2-2.9	
20-24	14	1.4	0.8-2.4	
+25	6	2.0	0.9-4.4	

Gravidity	N=2,345			0.709
1	23	1.8	1.2-2.7	
2	17	1.6	1.0-2.6	
Education	N=2,323			0.588
None	16	1.6	1.0-2.5	
Basic	20	2.0	1.3-3.1	
Secondary	4	1.3	0.5-3.4	
Socio-economic status	N=2,244			
Wealthiest	8	1.8	0.9-3.5	0.011
Wealthy	14	3.1	1.8-5.1	
Medium	2	0.4	0.1-1.7	
Poor	12	2.7	1.6-4.7	
Poorest	4	0.9	0.4-2.4	

6.5 Risk factors for a non-falciparum malaria infection during pregnancy detected at first antenatal clinic attendance by PCR.

Country of enrolment and the season of enrolment were the only variables associated significantly with the presence of non-falciparum malaria at first antenatal clinic attendance; age group, gravidity, educational and socioeconomic status were not significantly associated with non-falciparum malaria. In multivariate analysis (shown in table 43), the country and season of enrolment were entered into the model. The risk of women resident in Mali being infected with non-falciparum malaria was 23.9 times (95% CI 5.51-103.73, P<0.001) higher compared to women resident in the Gambia while that for women resident in Ghana was 5.02 times higher (95% CI 1.09-23.03, P=0.038) compared to the women enrolled in the Gambia. However, the confidence intervals for these analyses are large indicating the imprecision of these estimates due to small numbers of infected women. Women who were enrolled in the late wet season were 4.83 times (95% CI 1.87-12.43, P=0.001) more likely to have non-falciparum malaria compared to women enrolled in the early wet season.

Table 43: Risk of non-falciparum malaria infection at first antenatal clinic attendance as detected by PCR

Characteristic	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Country						
Gambia	1.00			1.00		
Burkina Faso	3.84	0.74-19.86	0.109	3.99	0.77-20.71	0.100
Ghana	5.37	1.17-24.60	0.030	5.02	1.09-23.03	0.038
Mali	18.85	4.42-80.36	<0.001	23.92	5.51-103.73	<0.001
Age group (years)						
>=25	1.00					
20-24	0.70	0.27-1.85	0.475			
<20	0.94	0.37-2.35	0.889			
Season of enrolment						
Early wet	1.00			1.00		
Late wet	2.92	1.19-7.26	0.019	4.83	1.87-12.43	0.001
Early dry	1.49	0.55-3.98	0.432	1.85	0.68-5.03	0.277
Late dry	1.85	0.75-4.55	0.181	1.70	0.68-4.21	0.254
Gravidity						
2	1.00					
1	1.13	0.60-2.12	0.709			
Education						
None	1.00					
Basic	1.31	0.67-2.54	0.424			
Secondary	0.83	0.27-2.49	0.735			
Socio-economic status						
Wealthiest	1.00					
Wealthy	1.77	0.73-4.26	0.203			
Medium	0.25	0.05-1.17	0.078			
Poor	1.57	0.64-3.89	0.326			
Poorest	0.52	0.16-1.75	0.293			

6.6 Characteristics of pregnant women with a malaria infection detected by microscopy at first antenatal clinic attendance and risk factors for infection by country

An analysis was undertaken to investigate whether there were any significant differences in the characteristics of women with a malaria infection detected by microscopy or in the risk factors for a microscopic infection by country. The characteristics of women who had a positive blood film at first antenatal clinic attendance are shown by country in Table 44.

With the exception of the Gambia, all the other three countries in the study showed inverse trends of decreasing prevalence with increasing age of the women. Malaria prevalence in the Gambia was much lower than in the other countries in the trial; also, the differences in the prevalence of malaria in the three age groups do not vary very much, with their confidence intervals overlapping greatly. The prevalence of malaria among the women aged less than 20 years was 10.2% (95% CI 7.4-14.0), decreasing to 6.6% (95% CI 4.2-10.1) among those aged 20-24 years, then increasing slightly to 7.1% (95% CI 3.0-16.2) among the oldest women. In all the four countries in the study, the primigravid women had a higher prevalence of malaria by microscopy compared to the secundigravid women. Among the women enrolled in Burkina Faso, Gambia and Ghana, the highest prevalence of malaria by microscopy was found in the women who had received only basic education. However, among the women resident in Mali, those who had had no education had a slightly higher prevalence even though the confidence intervals overlap. There is however, no clear pattern to the observation on the lowest prevalence; in Burkina Faso and Mali the lowest prevalence of malaria by microscopy was found among the women who had had secondary education or more while in Gambia and Ghana, the lowest prevalence was among those who had had no formal education. The highest prevalence of malaria by microscopy was found in the late wet season in Burkina Faso (75.0%, 95% CI 63.7-83.7) and Ghana (61.6%, 95% CI 53.8-68.9). In Mali (32.1%, 95% CI 22.8-43.1) and Gambia (15.1%, 95% CI 10.3-21.8) the highest prevalence was slightly later, occurring in the early dry season months of December to February. In Ghana (41.7%, 95% CI 34.4-49.3) and Mali (14.5%, 95% CI 9.9-20.7) the lowest prevalence was found in the late dry season months of March to May while in Burkina Faso (37.4%, 95% CI 29.5-46.0) and Gambia (5.6%, 95% CI 3.3-9.2) the lowest prevalence was in the early wet season months of June to August. In all the four countries in the study, the highest prevalence of malaria by microscopy was found among the poor or poorest women. In Burkina Faso (45.6%, 95% CI 38.5-52.9) and

Ghana (57.7%, 95% CI 49.0-65.9) the poorest women had the highest prevalence; in Gambia (14.1%, 95% CI 7.7-24.4) and Mali (47.1%, 95% CI 24.9-70.4) the poor women had the highest prevalence. With the exception of Burkina Faso, the lowest malaria prevalence by microscopy was found among the wealthiest women; in Burkina Faso, the women classified wealthy had the lowest prevalence (34.6%, 95% CI 18.8-54.7). In Mali, the three sub-sites were analysed separately; the San sub-site had the highest prevalence of malaria by microscopy (34.6%, 95% CI 28.1-41.6) while the Kita sub-site had the lowest prevalence of 7.3% (95% CI 3.9-13.0).

A number of risk factors were examined a priori to determine if they had an effect on malaria infection detected by microscopy among study women as shown in table 45.

In Burkina Faso, women who were less than 20 years old were 2.90 times (95% CI 1.14-7.38, P=0.026) more likely to have microscopic malaria compared to women 25 years or older. Similarly, women enrolled in Ghana who were less than 20 years old were 2.53 times (95% CI 1.46-4.40, P=0.001) more likely than women 25 years or older, to have microscopic malaria infection. In Gambia and Mali, age group was not a risk factor for microscopic malaria infection. Season of enrolment was a risk factor for microscopic malaria infection in all the four countries in the study. In Burkina Faso, women who were enrolled in the late wet season were 5.89 times (95% CI 2.86-12.11, P<0.001) more likely to have microscopic malaria compared to women enrolled during the early wet season. In Gambia women who were enrolled during the early dry season were 2.63 times (95% CI 1.27-5.43) more likely to have microscopic malaria compared to women enrolled during the early wet season. In Ghana women enrolled during the late wet season were 2.63 times (95% CI 1.66-4.15, P<0.001) more likely to have microscopic malaria when compared with women enrolled during the early wet season while women enrolled during the early dry season were also 1.61 times (95% CI 1.02-2.53, P=0.039) more likely to have microscopic malaria; women enrolled during the early dry

season in Mali were 2.26 times (95% CI 1.09-4.69, P=0.028) more likely to have microscopic malaria compared to those enrolled during the early wet season.

Primigravid women in Burkina Faso were 2.59 times (95% CI 1.52-4.42, P<0.001) more likely to have microscopic malaria compared to secundigravid women. A similar observation was made among Ghanaian women in the study where primigravid women were 1.57 times (95% CI 1.08-2.29, P=0.018) more likely to have microscopic malaria. Gravidity however, was not a risk factor for microscopic malaria among the Gambian and Malian women. The educational status of the women did not predict microscopic malaria in any of the countries in the study. With the exception of Burkina Faso, the socioeconomic status of the women was associated with microscopic malaria in the remaining countries. In Gambia, women who were poor were 3.63 times (95% CI 1.29-10.23, P=0.015) more likely to have microscopic malaria compared to the wealthiest women; women who were of medium socio-economic status were also 2.64 times (95% CI 1.04-6.71, P=0.041) more likely to have microscopic malaria. In Ghana, compared to the wealthiest women, all the socio-economic strata of women were more likely to have microscopic malaria; women who were poorest were 3.76 times (95% CI 1.94-7.30, P<0.001) more likely to have microscopic malaria. In Mali, women of medium socio-economic status were 2.56 times (95% CI 1.04-6.26, P=0.040) more likely to have microscopic malaria. Also in Mali, women enrolled at the San sub-site were 3.48 times (95% CI 1.49-8.10, P=0.004) more likely to have microscopic malaria compared to women enrolled at the Bamako suburban site.

Table 44: Characteristics of women with a malaria infection (all species) detected at first antenatal clinic attendance by microscopy by country.

Characteristic	Country							
	Burkina Faso		Gambia		Ghana		Mali	
	n(%)	95% CI	n(%)	95% CI	n(%)	95% CI	n(%)	95% CI
Age group(years)	(N=476)		(N=693)		(N=681)		(N=455)	
<20	110(53.9)	47.0-60.7	34(10.2)	7.4-14.0	144(67.0)	60.4-73.0	66(23.0)	18.5-28.2
20-24	83(36.2)	30.3-42.7	19(6.6)	4.2-10.1	140(43.9)	38.5-49.4	19(14.2)	9.2-21.2
25+	11(25.6)	14.6-40.8	5(7.1)	3.0-16.2	49(33.3)	26.2-41.4	4(11.8)	4.4-27.8
Gravidity	(N=476)		(N=693)		(N=678)		(N=455)	
1	126(55.8)	49.2-62.1	37(9.5)	7.0-12.9	207(58.0)	52.8-63.0	67(23.7)	19.1-29.0
2	78(31.2)	25.7-37.2	21(6.9)	4.5-10.4	124(38.6)	33.4-44.1	22(12.8)	8.6-18.7
Education	(N=472)		(N=675)		(N=681)		(N=454)	
None	145(41.8)	36.7-47.1	24(6.7)	4.5-9.8	62(41.3)	33.7-49.4	32(21.9)	15.9-29.4
Basic	41(56.2)	44.6-67.1	26(10.7)	7.4-15.3	232(53.0)	48.3-57.6	47(21.4)	16.4-27.3
Secondary	16(30.8)	19.7-44.7	7(9.6)	4.6-18.9	39(41.9)	32.3-52.2	10(11.4)	6.2-19.9
Season of enrolment	(N=476)		(N=693)		(N=681)		(N=455)	
Early wet	49(37.4)	29.5-46.0	14(5.6)	3.3-9.2	88(43.1)	36.5-50.1	29(16.5)	11.7-22.8
Late wet	54(75.0)	63.7-83.7	11(8.0)	4.5-14.0	98(61.6)	53.8-68.9	10(31.3)	17.5-49.3
Early dry	50(39.7)	31.5-48.5	23(15.1)	10.3-21.8	77(51.3)	43.3-59.3	26(32.1)	22.8-43.1
Late dry	51(34.7)	27.4-42.8	10(6.6)	3.6-11.8	70(41.7)	34.4-49.3	24(14.5)	9.9-20.7
Socio-economic status	(N=446)		(N=643)		(N=678)		(N=440)	
Wealthiest	6(42.9)	20.0-69.3	7(4.7)	2.3-9.6	24(27.6)	19.2-38.0	22(11.2)	7.5-16.5
Wealthy	9(34.6)	18.8-54.7	10(6.3)	3.4-11.3	43(48.9)	38.5-59.3	39(22.5)	16.9-29.4
Medium	54(48.2)	39.1-57.5	17(10.9)	6.9-16.9	68(49.3)	41.0-57.6	15(33.3)	21.1-48.4
Poor	43(38.4)	29.8-47.8	10(14.1)	7.7-24.4	120(51.1)	44.7-57.4	8(47.1)	24.9-70.4
Poorest	83(45.6)	38.5-52.9	12(11.2)	6.5-18.8	75(57.7)	49.0-65.9	3(33.3)	10.3-68.6
Sub-site							(N=455)	
Bamako sub-urban							13(10.3)	6.1-17.0
Kita							10(7.3)	3.9-13.0
San							66(34.6)	28.1-41.6

Table 45: Risk factors for malaria infection (all species) detected at first antenatal clinic attendance by microscopy by country; adjusted ORs are shown

Characteristic	Country											
	Burkina Faso			Gambia			Ghana			Mali		
	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Age group (years)												
>=25	1.00			1.00			1.00			1.00		
20-24	2.22	0.94-5.26	0.070	0.67	0.23-1.95	0.464	1.22	0.78-1.92	0.374	1.46	0.41-5.21	0.561
<20	2.90	1.14-7.38	0.026	0.86	0.28-2.60	0.788	2.53	1.46-4.40	0.001	2.88	0.86-9.71	0.087
Season of enrolment												
Early wet	1.00			1.00			1.00			1.00		
Late wet	5.89	2.86-12.11	<0.001	1.59	0.69-3.69	0.276	2.63	1.66-4.15	<0.001	2.57	0.93-7.10	0.069
Early dry	1.16	0.67-2.02	0.591	2.63	1.27-5.43	0.009	1.61	1.02-2.53	0.039	2.26	1.09-4.69	0.028
Late dry	0.80	0.47-1.38	0.423	1.06	0.45-2.50	0.891	0.98	0.63-1.52	0.913	1.19	0.61-2.31	0.608
Gravidity												
2	1.00			1.00			1.00			1.00		
1	2.59	1.52-4.42	<0.001	1.48	0.75-2.92	0.262	1.57	1.08-2.29	0.018	1.75	0.93-3.30	0.084
Education												
None	1.00			1.00			1.00			1.00		
Basic	1.67	0.92-3.00	0.089	1.50	0.81-2.76	0.197	1.32	0.86-2.00	0.201	0.88	0.48-1.62	0.683
Secondary	0.65	0.31-1.36	0.253	1.63	0.65-4.07	0.296	1.49	0.80-2.77	0.207	0.45	0.18-1.13	0.088
Socio-economic status												
Wealthiest	1.00			1.00			1.00			1.00		
Wealthy	0.34	0.07-1.58	0.168	1.45	0.53-3.98	0.470	2.35	1.20-4.58	0.012	1.27	0.65-2.47	0.488
Medium	0.71	0.19-2.68	0.616	2.64	1.04-6.71	0.041	2.37	1.27-4.44	0.007	2.56	1.04-6.26	0.040
Poor	0.51	0.13-1.94	0.319	3.63	1.29-10.23	0.015	2.76	1.52-5.02	0.001	2.68	0.78-9.15	0.177
Poorest	0.71	0.19-2.64	0.604	2.63	0.97-7.13	0.058	3.76	1.94-7.30	<0.001	1.73	0.31-9.59	0.528
Sub-site												
Bamako sub-urban										1.00		
Kita										0.36	0.13-1.02	0.055
San										3.48	1.49-8.10	0.004

6.7 Characteristics of women with a malaria infection detected by PCR at first antenatal clinic attendance and risk factors for infection by country

An analysis was undertaken to investigate whether there were any significant differences in the characteristics of women with a malaria infection detected by PCR or in the risk factors for an infection by country. The characteristics of women who had a positive PCR test at first antenatal clinic attendance are shown by country in table 46.

With the exception of Gambia, all the other countries in the study showed decreasing malaria prevalence by PCR with increasing age of the women. Among the women from Burkina Faso, women under 20 years of age had a prevalence of 72.9% (95% CI 66.4-78.6) decreasing to 39.5% (95% CI 26.0-54.9) among those aged 25 years and over; similar trends were observed among the Ghanaian and Malian women. However, in the Gambia, the highest prevalence was among the women aged 25 years and above (17.8%, 95% CI 10.6-28.4) while the lowest prevalence was among those aged 20-24 years (9.2%, 95% CI 6.4-13.0). Consistently, primigravid women had higher prevalence in all the countries in the study, than secundigravid women. In Burkina Faso, Ghana and Mali, women who had had basic education only had the highest prevalence of malaria while those who had received secondary education or more had the lowest prevalence. In the Gambia, women who had not received any formal education had the highest prevalence (14.3%, 95% CI 11.1-18.2). However, this was just slightly higher than those who had received basic education (14.1%, 95% CI 10.3-19.0) with overlapping confidence intervals; women who had received secondary education or more had the lowest prevalence in the Gambia (10.4%, 95% CI 5.4-20.0), as in the other countries in the study. In Burkina Faso (88.7%, 95% CI78.9-94.3) and Ghana (77.4%, 95% CI 70.2-83.2) the highest prevalence of malaria by PCR was observed during the late wet season while in the Gambia (18.3%, 95% CI 13.2-24.9) and Mali (51.2%, 95% CI 40.4-61.9) the highest prevalence was during the early dry season. In Burkina Faso (49.6%, 95% CI 41.1-58.2) and Mali (23.0%, 95%

CI 17.4-29.6) the lowest prevalence was during the early wet season; in Ghana (50.9%, 95% CI 43.4-58.4) the lowest prevalence was in the late dry season while in the Gambia (11.4%, 95% CI 7.1-17.8) this was in the late wet season. In Gambia (18.8%, 95% CI 12.5-27.1), Mali (66.7%, 95% CI 31.4-89.7) and Ghana (66.2%, 95% CI 60.0-72.0) the highest prevalence was found among the poor or poorest women in the study. In Burkina Faso the women with the highest prevalence belonged to the medium socio-economic group (64.6%, 95% CI 55.1-73.0). The lowest prevalence of malaria by PCR was found among the wealthiest women in Burkina Faso (50.0%, 95% CI 25.2-74.8), Ghana (40.2%, 95% CI 30.4-50.9), and Mali (18.3%, 95% CI 13.6-24.3) while in Gambia the wealthy women had the lowest prevalence (9.5%, 95% CI 5.9-15.0).

In Mali, women enrolled at the San sub-site had the highest prevalence (46.9%, 95% CI 40.0-54.0) while the women enrolled at the Bamako sub-urban sub-site had the lowest prevalence (16.9%, 95% CI 11.4-24.4).

Women who were less than 20 years old who were enrolled in Burkina Faso were 3.42 times (95% CI 1.43-8.21, P=0.006) more likely to have a positive malaria PCR compared to women who were 25 years or above (table 47). Similar findings were obtained in Ghana (adjusted OR 2.63, 95% CI 1.49-4.66, P=0.001) and Mali (adjusted OR 2.59, 95% CI 1.02-6.59, P=0.046). However, in the Gambia women who were less than 20 years old were 57% (adjusted OR 0.43, 95% CI 0.19-0.95, P=0.037) less likely to have a positive malaria PCR while women aged 20-24 years were also 68% (adjusted OR 0.32, 95% CI 0.15-0.68, P=0.003) less likely to have a positive malaria PCR when compared with women 25 years and above. In the Gambia, the season of enrolment was not associated with positive malaria PCR. However in the other three countries in the study, positive malaria PCR was associated with season of enrolment. In Burkina Faso, women enrolled in the late wet season were 9.92 times (95% CI 4.02-24.44, P<0.001) more likely to have a positive malaria PCR compared to women enrolled in the early

wet season; in the same country, women enrolled in the early dry season were also 2.08 times (95% CI 1.20-3.63, P=0.009) more likely to have a positive malaria PCR. Findings similar to those in Burkina Faso were obtained in Ghana; women enrolled in the late wet season were 4.14 times (95% CI 2.52-6.79, P<0.001) more likely to have a positive malaria PCR while women enrolled in the early dry season were 2.10 times (95% CI 1.32-3.33, P=0.002) more likely to have a positive malaria PCR. In Mali, women enrolled in the early dry season were 3.19 times (95% CI 1.70-5.98, P<0.001) more likely to have a positive malaria PCR compared to women enrolled during the early wet season.

Gravidity was a risk factor for positive malaria PCR only in Burkina Faso. Primigravid women there were 2.12 times (95% CI 1.24-3.64, P=0.006) more likely than secundigravid women to have a positive malaria PCR. Educational status was not associated with positive malaria PCR in Gambia, Ghana and Mali. However, in Burkina Faso, women who had received basic education were 1.96 times (95% CI 1.03-3.75, P=0.041) more likely to have positive malaria PCR when compared to women with no formal education.

Socio-economic status was not associated with positive malaria PCR in Burkina Faso and Gambia. In Ghana, the poorest women were 3.01 times (95% CI 1.57-5.78, P=0.001) more likely to have positive malaria PCR compared to the wealthiest women; the poor women were also 3.19 times (95% CI 1.78-5.73, P<0.001) more likely to have positive malaria PCR. Similarly, in Mali, the poorest women were 6.71 times (95% CI 1.44-31.39, P=0.016) more likely to have positive malaria PCR compared to the wealthiest women. Also in Mali, women enrolled at the San sub-site were 2.73 times (95% CI 1.37-5.44, P=0.004) more likely to have positive malaria PCR compared to women enrolled at the Bamako sub-urban sub-site.

Table 46: Characteristics of women with a malaria infection (all species) detected at first antenatal clinic attendance by PCR by country

Characteristic	Country							
	Burkina Faso		Gambia		Ghana		Mali	
	n(%)	95% CI	n(%)	95% CI	n(%)	95% CI	n(%)	95% CI
Age group(years)	(N=474)		(N=725)		(N=683)		(N=464)	
<20	148(72.9)	66.4-78.6	58(16.7)	13.1-21.0	167(77.0)	70.9-82.1	100(34.0)	28.8-39.6
20-24	120(52.6)	46.1-59.1	28(9.2)	6.4-13.0	176(55.2)	49.7-60.6	37(27.6)	20.7-35.8
25+	17(39.5)	26.0-54.9	13(17.8)	10.6-28.4	69(46.9)	39.0-55.1	8(22.2)	11.4-38.9
Gravidity	(N=474)		(N=725)		(N=680)		(N=464)	
1	161(71.9)	65.6-77.4	65(15.9)	12.7-19.8	242(67.4)	62.4-72.1	94(32.9)	27.7-38.6
2	124(49.6)	43.4-55.8	34(10.8)	7.8-14.7	168(52.3)	46.9-57.8	51(28.7)	22.5-35.8
Education	(N=470)		(N=705)		(N=683)		(N=463)	
None	204(59.1)	53.8-64.2	54(14.3)	11.1-18.2	81(54.0)	46.0-61.9	48(31.6)	24.7-39.4
Basic	53(72.6)	61.2-81.7	35(14.1)	10.3-19.0	283(64.3)	59.7-68.7	75(33.6)	27.7-40.1
Secondary	25(48.1)	34.1-61.6	8(10.4)	5.4-20.0	48(51.6)	41.5-61.6	22(25.0)	17.0-35.2
Season of enrolment	(N=474)		(N=725)		(N=683)		(N=464)	
Early wet	65(49.6)	41.1-58.2	34(13.1)	9.5-17.8	105(51.2)	44.4-58.0	42(23.0)	17.4-29.6
Late wet	63(88.7)	78.9-94.3	16(11.4)	7.1-17.8	123(77.4)	70.2-83.2	13(39.4)	24.2-57.0
Early dry	83(65.9)	57.1-73.7	31(18.3)	13.2-24.9	98(65.3)	57.3-72.6	42(51.2)	40.4-61.9
Late dry	74(50.7)	42.6-58.8	18(11.6)	7.4-17.7	86(50.9)	43.4-58.4	48(28.9)	22.5-36.3
Socio-economic status	(N=444)		(N=669)		(N=680)		(N=449)	
Wealthiest	7(50.0)	25.2-74.8	16(10.4)	6.5-16.3	35(40.2)	30.4-50.9	37(18.3)	13.6-24.3
Wealthy	14(53.9)	34.7-72.0	16(9.5)	5.9-15.0	50(56.8)	46.2-66.8	66(37.5)	30.6-44.9
Medium	71(64.6)	55.1-73.0	30(18.6)	13.3-25.4	83(60.1)	51.7-68.0	22(48.9)	34.6-63.4
Poor	63(56.3)	46.9-65.2	12(16.2)	9.4-26.5	157(66.2)	60.0-72.0	10(58.8)	34.5-79.5
Poorest	113(62.1)	54.8-68.9	21(18.8)	12.5-27.1	84(64.6)	56.0-72.4	6(66.7)	31.4-89.7
Sub-site							(N=464)	
Bamako sub-urban							22(16.9)	11.4-24.4
Kita							31(22.1)	16.0-29.8
San							91(46.9)	40.0-54.0

Table 47: Risk factors for malaria infection (all species) detected at first antenatal clinic attendance by PCR by country; adjusted ORs are shown

Characteristic	Country											
	Burkina Faso			Gambia			Ghana			Mali		
	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Age group (years)												
>=25	1.00			1.00			1.00			1.00		
20-24	2.00	0.91-4.39	0.083	0.32	0.15-0.68	0.003	1.15	0.74-1.79	0.530	1.41	0.54-3.67	0.485
<20	3.42	1.43-8.21	0.006	0.43	0.19-0.95	0.037	2.63	1.49-4.66	0.001	2.59	1.02-6.59	0.046
Season of enrolment												
Early wet	1.00			1.00			1.00			1.00		
Late wet	9.92	4.02-24.44	<0.001	0.87	0.45-1.67	0.667	4.14	2.52-6.79	<0.001	1.98	0.83-4.73	0.123
Early dry	2.08	1.20-3.63	0.009	1.48	0.84-2.59	0.173	2.10	1.32-3.33	0.002	3.19	1.70-5.98	<0.001
Late dry	1.04	0.62-1.75	0.885	0.77	0.41-1.47	0.433	1.00	0.64-1.54	0.987	1.70	0.98-2.94	0.057
Gravidity												
2	1.00						1.00			1.00		
1	2.12	1.24-3.64	0.006	1.70	0.96-2.97	0.061	1.39	0.94-2.04	0.097	1.02	0.62-1.68	0.945
Education												
None	1.00			1.00			1.00			1.00		
Basic	1.96	1.03-3.75	0.041	0.87	0.54-1.42	0.583	1.31	0.86-2.01	0.213	1.00	0.59-1.69	0.996
Secondary	0.84	0.42-1.71	0.638	0.72	0.32-1.63	0.433	1.40	0.75-2.60	0.294	0.88	0.43-1.79	0.717
Socio-economic status												
Wealthiest	1.00			1.00			1.00			1.00		
Wealthy	0.66	0.14-2.99	0.590	0.87	0.42-1.82	0.705	1.92	1.00-3.68	0.051	1.72	1.01-2.92	0.045
Medium	1.25	0.34-4.67	0.737	1.89	0.97-3.70	0.062	2.19	1.19-4.02	0.012	2.95	1.37-6.32	0.005
Poor	0.96	0.25-3.60	0.947	1.67	0.73-3.81	0.220	3.19	1.78-5.73	<0.001	3.44	1.08-10.96	0.037
Poorest	1.25	0.34-1.09	0.741	2.09	1.01-4.33	0.048	3.01	1.57-5.78	0.001	6.71	1.44-31.39	0.016
Sub-site												
Bamako sub-urban										1.00		
Kita										0.77	0.37-1.61	0.484
San										2.73	1.37-5.44	0.004

6.8 Summary of findings

This chapter presents prevalence of falciparum and non-falciparum malaria detected by microscopy or PCR among 2,348 women who were enrolled into the trial of IPT versus IST. The distribution of women in the sample was not uniform. Women from Gambia constituted 30.8% of the sample, with 29.1% from Ghana, 20.3% from Burkina Faso and 19.8% from Mali.

The analysis showed that 39.01% of the women in this sub-sample had malaria infection with majority of these being *P. falciparum* infections (38.38%) while only 1.70% of the women had non-falciparum infections either as mono infections or as mixed infections with *P. falciparum*. Two women (0.09%) had triple species infection (*P. falciparum, P. malariae and P. ovale curtisi*). The analysis also showed that 13.1% of the malaria infections detected by PCR were not detected by RDT. Out of 707 women who had falciparum malaria detected by PCR, 12.6% were missed by the RDT used. Most of the non-falciparum malaria mono infections detected by PCR were not detected by RDT. However, the majority of the mixed infections were detected by the RDT used. The highest prevalence of non-falciparum malaria was found among the women enrolled in Mali (5.0%) while the lowest was in Gambia (0.3%). The risk factors of non-falciparum malaria among the women in the study were country of residence and season of enrolment.

The prevalence of malaria detected by microscopy ranged from a minimum of 8.4% in Gambia to a maximum of 48.9% in Ghana. Significant risk factors of malaria infection detected by microscopy were the country of residence, age group, season of enrolment, gravidity educational status and socio-economic status.

The prevalence of malaria detected by diagnostic PCR tests on blood spots ranged from a minimum of 13.7% in Gambia to a maximum of 60.3% in Ghana. The risk factors of malaria detected by

PCR among the women in the study were similar to those for malaria detected by microscopy except for educational status which was not associated with malaria detected by PCR.

CHAPTER SEVEN

The ability of Symptoms to Predict Malaria in Pregnancy

7.1 Introduction

As many countries reach the point where they need to consider alternatives to IPTp with Sulfadoxine-Pyrimethamine, considerations will be given to intermittent screening and treatment (ISTp) as an option for adoption as a replacement policy. This will mean that all women attending ANC will undergo rapid diagnostic testing for malaria routinely. This procedure will require more of the time of health workers than is required to administer IPTp. In many developing countries health facilities are often under-staffed and the few health workers available are often over-worked and poorly-motivated and have to contend with having to perform several procedures for each woman attending ANC. In addition, the cost of rapid diagnostic testing also results in a marginal increase in the cost of ANC even though RDTs have become increasingly cheaper with time. The increased work load, and the cost of IST, could be reduced if it was possible identify women in whom screening was essential and others in whom it was not, as their risk of malaria was low. This chapter presents the results of an analysis of the ability of a set of symptoms solicited from pregnant women in the study each time they came to the clinic on a scheduled visit, to predict the likelihood that they might have a malaria infection. In view of the fact that the RDT screening was conducted only for the women in the IST arm of the study, the analysis is limited to this group of women. At scheduled clinic visits, the women were asked whether they had any of 15 symptoms listed on a checklist. The symptoms solicited on the first and second visits only were used in this analysis because of significant reduction in the number of women attending clinics at subsequent visits as well as the paucity of symptoms obtained from the women at later visits. In subsequent

sections of this chapter, the distribution of symptoms is presented followed by an analysis of which symptoms were associated with a positive RDT. Symptom scores were then created from symptoms which were associated with positive RDT in univariate logistic regression analysis. Multivariate logistic regression modeling was then used to establish the strength of the association between the symptom scores and positive RDT, controlling for some background characteristics of the study participants.

7.2 Symptoms recorded frequently at antenatal clinic visits

Table 48 shows the distribution of various symptoms solicited from women on their first and second study visits. The commonest symptom that the women had on the day of enrolment was headache (17.6%). During the second study visit, 13.0% of the women said that they were 'currently sick' and this was the commonest complaint. Other symptoms complained of on the first study visit were being easily tired (15.1%), currently sick (14.4%), lower abdominal pain (14.3%) and waist pain (11.0%). The least common symptom on the day of enrolment was diarrhoea (0.8%).

During the second visit, in addition to many reporting that they were currently sick, headache was reported by 12.1% of the women. The least common complaint was again diarrhoea (1.1%).

Table 48: Distribution of symptoms solicited at the first two study visits

Symptom	**First visit (v0)** **n (%)**	**Second visit (v1)** **n (%)**
Currently sick	188(14.4)	144(13.0)
Fever	67(5.1)	42(3.8)
Chills	66(5.1)	34(3.1)
Headache	230(17.6)	134(12.1)
Nausea	48(3.7)	21(1.9)
Vomiting	35(2.7)	21(1.9)
Diarrhoea	11(0.8)	12(1.1)
General weakness	98(7.5)	57(5.1)

Dizziness	107(8.2)	47(4.2)
Easily tired	197(15.1)	48(4.3)
General malaise	73(5.6)	27(2.4)
Lower abdominal pain	187(14.3)	70(6.3)
Painful urination	96(7.4)	17(1.5)
Waist pain	144(11.0)	87(7.8)
Cough	75(5.7)	61(5.5)
Total (N)	**1,306**	**1,111**

7.3 Symptoms associated with a positive RDT result

At the first visit, four symptoms were significantly associated with a positive RDT using a significance level of P< 0.100. These symptoms were fever, chills, headache and waist pain. Table 49 shows the proportions of women presenting with various symptoms who had positive or negative RDT results at both the first and second visits.

Of the women who admitted to having fever on the first study visit, 73.5% (95% CI 56.1-85.8) had a positive RDT result compared to 57.4% (95% CI 53.5-61.3) of the women who had no fever who also tested positive. For the women who had chills, 75.8% (95% CI 58.2-85.8) of them tested positive by RDT while 57.4% (95% CI 53.4-61.2) of those who did not have chills also tested positive by RDT. For the women who had headache, 66.7% (95% CI 58.0-74.4) of them tested positive by RDT compared to 56.3% (95% CI 52.0-60.5) of women who did not report headache who also tested positive by RDT. Of the women who had waist pain, 68.7% (95% CI 57.9-77.8) of them had positive RDT results while 56.8% (95% CI 52.7-60.8) of those who had no waist pain also had positive RDT.

At the second visit, 9 of the solicited symptoms including all of the four symptoms observed at the first visit, were associated with positive RDT results. In addition to fever, chills, headache and waist pain, vomiting, generalised weakness, dizziness, painful urination and being currently sick

were found to be associated with a positive RDT. The proportions of women who had various symptoms who also tested positive by RDT at the second visit are shown in table 7.2.

Table 50 shows univariate logistic regression results using RDT result as the dependent variable, with the various symptoms as independent variables to establish whether there is any association between these symptoms and a positive RDT result.

Table 49: Table showing proportion of women presenting specific symptoms and their RDT results

Symptom	**First visit (v0)**			**Second visit (v1)**		
	RDT +ve(%)	**95% CI**	**P-value**	**RDT +ve**	**95% CI**	**P-value**
Currently sick	(N=652)		0.279	(N=564)		0.027
Yes	65(63.1)	53.3-71.9		38(45.8)	35.3-56.6	
No	315(57.4)	53.2-61.5		160(33.3)	29.2-37.6	
Fever	(N=652)		0.064	(N=564)		0.013
Yes	25(73.5)	56.1-85.8		15(57.7)	38.1-75.1	
No	355(57.4)	53.5-61.3		183(34.0)	30.1-38.1	
Chills	(N=652)		0.037	(N=564)		0.031
Yes	25(75.8)	58.2-87.5		12(57.1)	35.4-76.4	
No	355(57.4)	53.4-61.2		186(34.3)	30.4-38.4	
Headache	(N=652)		0.034	(N=564)		<0.001
Yes	84(66.7)	58.0-74.4		41(54.0)	42.6-64.9	
No	296(56.3)	52.0-60.5		157(32.2)	28.2-36.5	
Vomiting	(N=652)		0.577	(N=564)		0.021
Yes	11(52.4)	31.4-72.6		8(66.7)	36.3-87.5	
No	369(58.5)	54.6-62.3		190(34.4)	30.6-38.5	
Generalised weakness	(N=652)		0.385	(N=564)		0.036
Yes	29(64.4)	49.4-77.1		15(53.6)	35.1-71.1	
No	351(57.8)	53.8-61.7		183(34.1)	30.2-38.3	
Dizziness	(N=652)		0.672	(N=564)		0.036

Yes	34(55.7)	43.1-67.7		15(53.6)	35.1-71.1	
No	346(58.5)	54.5-62.5		183(34.1)	30.2-38.3	
Painful urination	(N=652)		0.232	(N=564)		0.001
Yes	35(66.0)	52.3-77.6		8(88.9)	46.8-98.7	
No	345(57.6)	53.6-61.5		190(34.2)	30.4-38.3	
Waist pain	(N=652)		0.040	(N=564)		0.015
Yes	57(68.7)	57.9-77.8		25(51.0)	37.1-64.8	
No	323(56.8)	52.7-60.8		173(33.6)	29.6-37.8	

Table 50: Table showing univariate logistic regression results for various symptoms using RDT result as the dependent variable

Symptom	**First visit (v0)**			**Second visit (v1)**		
	Crude OR	**95% CI**	**P-value**	**Crude OR**	**95% CI**	**P-value**
Currently sick	1.27	0.82-1.96	0.280	1.69	1.05-2.72	0.028
Fever	2.05	0.94-4.48	0.069	2.65	1.19-5.88	0.017
Chills	2.32	1.03-5.23	0.042	2.56	1.06-6.18	0.037
Headache	1.55	1.03-2.34	0.034	2.47	1.51-4.03	<0.001
Vomiting	0.78	0.33-1.87	0.578	3.81	1.13-12.82	0.031
General weakness	1.32	0.70-2.49	0.386	2.23	1.04-4.78	0.040
Dizziness	0.89	0.52-1.52	0.672	2.23	1.04-4.78	0.040
Painful urination	1.43	0.79-2.59	0.234	15.37	1.91-123.79	0.010
Waist pain	1.67	1.02-2.73	0.041	2.06	1.14-3.71	0.016

7.4 Association of symptom scores with positive RDT

Symptoms which were found to be associated with positive RDT in univariate analyses were used to construct simple symptom scores to determine if their predictive power for the presence of malaria in pregnancy could be used in determining women who require screening for malaria at antenatal clinics as a priority. Two scores were constructed using symptoms found to be associated with a positive RDT at the first two study visits.

The first score (score 1) used the four symptoms (fever, chills, headache and waist pain) detected at the first study visit (v0) to be associated with a positive RDT in univariate analyses. Table 51 shows the distribution of study participants on the symptom score by RDT positivity. There is a linear trend with increasing proportions of RDT positive with increasing number of symptoms present. Of the women who had no symptoms, 55.3% (95% CI 50.7-59.7) had a positive RDT result compared to 85.0% (95% CI 61.6-95.2) of the women who had three or four symptoms present.

The second score used the nine symptoms which were found during the second study visit to have an association with a positive RDT. These include being currently sick, fever, chills, headache, waist pain, vomiting, generalised weakness, dizziness and painful urination. Owing to small numbers in some categories, the score was divided into four categories; no symptoms, one symptom, two symptoms and 3 to 8 symptoms. Of the women who had no symptoms, 31.5% (95% CI 27.2-36.0) of them had a positive RDT while 65.2% (95% CI 50.4-77.6) of those who had 3 to 8 symptoms had a positive RDT.

Table 51: Symptom score by RDT result

Symptom Score	**RDT Positive n (%)**	**95% CI**	**P-value**
Score 1			0.018
No symptoms	258(55.3)	50.7-59.7	
1 symptom	75(61.2)	53.0-70.2	
2 symptoms	30(68.2)	53.0-80.3	
3 or 4 symptoms	17(85.0)	61.6-95.2	
Score 2			<0.001
No symptoms	134(31.5)	27.2-36.0	
1 symptom	17(38.6)	25.4-53.8	
2 symptoms	17(35.4)	23.2-49.9	
3 to 8 symptoms	30(65.2)	50.4-77.6	

Table 52 shows the results of regression analyses conducted with the two scores independently in different models as predictors and RDT result for the specified visit as the dependent variable. Multivariate analyses controlled for age group, educational status, gravidity, socio-economic status and season of enrolment in each of the models.

For score 1, women who had 3 or 4 symptoms were 3.87 times (95% CI 1.07-14.09, P=0.040) more likely to have a positive RDT when compared with women who had no symptoms adjusting for age group, educational status, gravidity, socio-economic status and season of enrolment.

For score 2, women who had 3 to 8 symptoms were 3.43 times (95% CI 1.57-7.45, P=0.002) more likely to have a positive RDT compared to women who had no symptoms adjusting for the same covariates as with score 1.

Table 52: Association between symptom scores and RDT results

Symptom score	Crude OR	95% CI	P-value	Adjusted OR*	95% CI	P-value
Score 1						
No symptom	1.00			1.00		
1 symptom	1.32	0.88-1.99	0.183	1.37	0.87-2.14	0.169
2 symptoms	1.73	0.90-3.36	0.102	1.73	0.84-3.55	0.137
3 or 4 symptoms	4.59	1.33-15.88	0.016	3.87	1.07-14.09	0.040
Score 2						
No symptom	1.00			1.00		
1 symptom	1.37	0.72-2.60	0.333	1.35	0.63-2.91	0.435
2 symptoms	1.19	0.64-2.23	0.577	1.03	0.50-2.14	0.933
3 to 8 symptoms	4.09	2.15-7.75	<0.001	3.43	1.57-7.45	0.002

*Adjusted for age group, educational status, gravidity, socio-economic status and season of enrolment.

7.5 Sensitivity and specificity of Symptom scores

To estimate the usefulness of these symptom scores in determining the necessity to conduct rapid malaria diagnostic testing when resources are limited, it was necessary to calculate the sensitivity and specificity of these scores using RDT tests as the gold standard. To enable this to be done, it was necessary to re-categorise the scores into dichotomous variables to allow 2x2 tables to be created.

7.5.1 Sensitivity and specificity of Ma1_score

Ma1_score was originally categorized into 4 levels: no symptom, 1 symptom, 2 symptoms and 3 or 4 symptoms. In re-categorising this score, two options were tried. The first option is referred to as Ma1_score1 which dichotomises Ma1_score into: Positive (1) which refers to women who had any symptom and Negative (0) which refers to women who had no symptom. Table 53 shows a cross-tabulation of Ma1_score1 versus RDT result for visit 0. The sensitivity of this score is 27.4% (95% CI 32.1, 37.1) while the specificity is 71.4% (95% CI 76.8, 81.7).

Table 53: Ma1_score1 vs RDT Visit 0

Ma_1 Score1	**RDT visit 0**		
	Positive	**Negative**	**Total**
Positive (1)	122	63	185
Negative (0)	258	209	467
Total	380	272	652

Sensitivity: 27.4% (95% CI 32.1, 37.1)

Specificity: 71.4% (95% CI 76.8, 81.7)

PPV: 65.8% (95% CI 58.6, 72.7

NPV: 44.8% (95% CI 40.2, 49.4)

The second option which is referred to here as Ma1_score2 re-categorises Ma1_score into: Positive (1) which refers to women who had 3 or 4 symptoms and Negative (0) which refers to women who had 1 or 2 or no symptoms at all. Table 54 shows the cross-tabulation of Ma1_score2 with results of RDT visit 0. The sensitivity of this score reduced considerably to 4.5% (95% CI 2.6, 7.1) while the specificity increased to 98.9% (95% CI 96.8, 99.8).

Table 54: Ma1_score2 versus RDT visit 0

Ma_1 Score2	**RDT visit 0**		
	Positive	**Negative**	**Total**
Positive (1)	17	3	20
Negative (0)	363	269	632
Total	380	272	652

Sensitivity: 4.5% (95% CI 2.6, 7.1)

Specificity: 98.9% (95% CI 96.8, 99.8)

PPV: 85.0% (95% CI 62.1, 96.8)

NPV: 42.6% (95% CI 38.7, 46.5)

7.5.2 Sensitivity and specificity of Ma2_score

Ma2_score was initially categorised into four levels: no symptom, 1 symptom, 2 symptoms and 3 to 8 symptoms. In creating a dichotomous variable, Ma2_score two options were explored. The first option created Ma2_score1 which has two outcomes: Positive (1) which refers to women who had one or more symptoms and Negative (0), which refers to women who had no symptoms. Table 55 shows the cross-tabulation of Ma2_score1 with RDT for visit 1 result. The sensitivity of Ma2_score1 was 32.3% (95% CI 25.8, 39.3) while the specificity was 79.8% (95% CI 75.3, 83.8).

Table 55: Ma2_score1 versus RDT visit 1

Ma_2 Score1	RDT visit 1		
	Positive	**Negative**	**Total**
Positive (1)	64	74	198
Negative (0)	134	292	366
Total	198	366	564

Sensitivity: 32.3% (95% CI 25.8, 39.3)

Specificity: 79.8% (95% CI 75.3, 83.8)

PPV: 46.4% (95% CI 37.9, 55.1)

NPV: 68.5% (95% CI 63.9, 72.9)

The second option created Ma2_score2 which also has two outcomes: Positive (1) refers to women who had 3 to 8 symptoms while Negative (0) groups women who either had no symptoms or had one or two symptoms. Table 56 shows the cross-tabulation between Ma2_score2 and RDT visit 1 results. The sensitivity of Ma2_score2 using RDT visit 1 results as the gold standard was 15.2% (95% CI 10.5, 20.9) while the specificity was 95.6% (95% CI 93.0, 97.5).

Table 56: Ma2_score2 versus RDT visit 1

Ma_2 Score2	RDT visit 1		
	Positive	**Negative**	**Total**
Positive (1)	30	16	46
Negative (0)	168	350	518
Total	198	366	564

Sensitivity: 15.2% (95% CI 10.5, 20.9)

Specificity: 95.6% (95% CI 93.0, 97.5)

PPV: 65.2% (95% CI 49.8, 78.6)

NPV: 67.6% (95% CI 63.3, 71.6)

7.6 Summary

Nine symptoms were associated with a positive RDT result in initial analysis of symptoms solicited at the first two ANC visits. These were being currently sick, fever, chills, headache, waist pain, vomiting, dizziness, generalised weakness and painful urination. Two symptom scores were constructed with the data from the two visits to establish whether these scores could predict the need for RDT testing in a situation where everyone cannot be tested and decisions have to be made about who to test. The analyses indicated that of the nine symptoms mentioned above the presence of three or more of them was more than three times likely to result in a positive RDT compared to women who had no symptoms at all.

However, analyses of the sensitivities of the scores using the results of the RDT tests as the gold standard indicate that the sensitivity of the scores are very low even though their specificities are much higher. Therefore their usefulness in deciding which women should benefit from RDT testing in the event that resources for testing are not available for all who require testing, is very limited.

CHAPTER EIGHT

Discussion and Conclusion

8.1 Introduction

This chapter discusses the findings of this study in the light of currently available literature and examines the implications of these findings for policy, practice and further research. The order of discussion in this chapter follows the order in which the results were presented in the earlier chapters.

8.2 Sensitivity and Specificity of RDT in the Diagnoses of Placental malaria

First Response® Combo RDT

A major consequence of placental malaria is low birth weight resulting from both prematurity and intrauterine growth retardation (Steketee et al. 2001; Bouvier et al. 1997). It is estimated that annually as many as 75,000-200,000 infant deaths worldwide, are linked to malaria in pregnancy (Dellicour et al. 2010; Desai et al. 2007; Steketee et al. 2001). This heavy mortality toll makes it imperative that efforts are made to enhance the early diagnosis of malaria in pregnancy and by extension placental malaria. Even though placental histology is often regarded as the gold standard for epidemiological research in malaria in pregnancy, its usefulness to the individual woman and her baby who have suffered the consequences of placental malaria is almost nil as the placenta can only be examined post-partum. Therefore laboratory tests which could accurately detect malaria in pregnancy leading to appropriate case-management may help to prevent placental malaria and its consequences.

In this study, only active placental malaria infections were considered i.e. where malaria parasites were present in the placenta with or without malaria pigment. Women with malaria pigment only

in the placenta, indicative of past malaria infections were classified with those with no infection. In this trial, the prevalence of malaria infection among the women was very high, particularly during the first study visit. Therefore, most of the women in the study had experienced malaria infection sometime in the course of the pregnancy even though most would have been detected at some time in the course of the pregnancy and treated. Therefore the incidence of past placental infection was very high. However, even though past infection is a clinically relevant outcome (Kattenberg et al. 2011) and indicates that the participant has been infected during the course of the pregnancy, for the purpose of assessing the sensitivity of a diagnostic test such as an RDT, the presence of parasites as in active infections, with or without pigment is the most important factor even though in excluding past infections, one underestimates the incidence of placental malaria. The purpose of this analysis was to find out if rapid malaria diagnostic testing could adequately predict the presence of placental malaria. It has been established that *Plasmodium falciparum* parasites tend to sequestrate in the intervillous bed of the placenta and their presence in the placenta is not necessarily reflected by peripheral malaria parasitaemia. Accordingly, blood smear microscopy which detects peripheral malaria parasitaemia, when negative, may miss placental malaria. However, RDTs which depend on the detection of either histidine-rich protein 2 (HRP-2), plasmodium lactate dehydrogenase (pLDH) or aldolase, the last two being enzymes in the glycolytic pathway of the malaria parasite, could theoretically be detected in peripheral blood even though they are being produced by malaria parasites sequestered in the placental bed. The RDT used in this study (First Response® Combo RDT) contained test reagents to detect HRP-2, (which can only detect *P. falciparum*) as well as reagents to detect pLDH which can detect the presence *P. vivax, P. malariae* and *P. ovale* as well as *P. falciparum*. The test strip has three bands; a control band, a band for P. falciparum only and another band for detecting any of the four plasmodium

species mentioned above (PAN). The First Response® Malaria pLDH/HRP2 Combo test is designed for the differential diagnosis between P. falciparum and other plasmodium species. However, it is unable to differentiate between the three different non-falciparum species which it captures on one test band.

The sensitivity of the RDT to detect placental malaria at scheduled and unscheduled visits was 63.1% (95% CI 55.9-69.9) while that for the scheduled visits alone was 72.0% (95% CI 63.3-79.7). This finding of sensitivity being higher among a mixture of women who were attending scheduled and unscheduled visits compared to women who were attending unscheduled visits and were therefore unwell, at initial glance may seem unexpected and implausible as one would usually expect that women who are sick are likely to have peripheral parasitaemia and therefore the sensitivity of the RDT would be expected to be higher among them than for women who are not sick. However, placental malaria is not always associated with systemic illness as the parasites are sequestered in the placenta and may not necessarily be found in peripheral blood where most of the clinical signs emanate from. Accordingly, the finding of higher sensitivity among women attending both scheduled and unscheduled visits may reflect the fact that even during scheduled visits parasites may be present in the placental bed and not necessarily producing any obvious clinical effects. It is also possible that most of the infections that could be detected by RDT were detected at first visit and as expected, and also found in this study, there were fewer infections in later visits.

The sensitivity of the RDT to detect placental malaria was estimated for the RDTs done at various visits. This was done on the assumption that the women were exposed to malaria infections from the time of enrolment or earlier, till they delivered and therefore the time when the placental

malaria began could not be determined exactly. The sensitivity of the RDT conducted at various visits to detect placental malaria was: first visit: 55.7% (95% CI 46.5-64.7), second visit: 39.1% (95% CI 25.0-34.0), third visit: 33.3% (95% CI 23.4-44.5) and delivery visit: 35.4% (95% CI 26.6-45.0). This shows a pattern of declining sensitivity with increasing gestational age, with the exception of the sensitivity of the RDT at delivery which is slightly higher than that of the third visit, which may not be significant at their confidence intervals overlap to a large extent. It is likely that as a pregnancy progresses the woman develops some level of immunity against malaria infection. This leads to an increase in the frequency of low-density infections as was found in this study and consequently lower sensitivity as pregnancy progresses (Williams et al. 2016).

The major limitation of estimating the sensitivity of RDT at different ANC visit and the cumulative sensitivity of RDT done all ANC visits stems from the fact that women who tested positive to an RDT at any time of the study were given a course of Artemether Lumefantrine to treat the malaria infection and therefore even though these infections were detected they were all treated and if the treatment given was successful, those infections should not progress to the point of being detected as active infections in the placenta at delivery. The RDTs that were done at specified study visits just provide information about malaria infection at a point in time and do not provide adequate explanation for active placental malaria which could have begun after a particular study visit being referred to. Despite these limitations, studies have been conducted to try to establish whether rapid malaria diagnostic tests could detect placental malaria, with equivocal outcomes.

In a study in eastern Sudan in an area of unstable malaria transmission, Kashif et al. (2013) testing a malaria RDT, the SD Bioline P.f/P.v (Bio Standard Diagnostics, Gurgaon, Korea), an HRP2 and *P. vivax* lactate dehydrogenase RDT, for its ability to detect placental malaria, found that the RDT

had a sensitivity of 17.4% (95% CI 8.4-30.4) and a specificity of 81.7% (95% CI 73.4-88.3) using placental histology as the gold standard. The authors concluded that the RDT performed poorly in the detection of placental malaria in their setting. In another study to assess the impact of HIV and malaria co-infection on mother-to-child HIV transmission and adverse birth outcomes in Uganda the authors obtained a sensitivity of 57% for RDT in peripheral blood using placental histology combined with HRP2 immunohistochemistry as gold standard. The RDT used in this study was the Binax NOW ICT (Binax, Portland, ME) which detects *P. falciparum*-specific HRP2 and pan-Plasmodium aldolase antigens (Brahmbhatt et al. (2008).

In a study conducted retrospectively with samples collected at delivery from pregnant women participating in a placebo-controlled trial with sulfadoxine-pyrimethamine in Manhica in southern Mozambique, an area of perennial malaria transmission with some seasonality mostly attributable to *P. falciparum,* the investigators, using the HRP2-based Malaria Antigen P.f. rapid diagnostic test from SD Bioline (Standard Diagnostics) obtained a sensitivity of 76% using placental histology as the reference standard (Mayor et al. 2012). A limitation of this study however, is that whole blood was not used as recommended by the manufacturer. The tests were performed on archived plasma samples, which may have affected the results of these tests.

8.3 Sensitivity and specificity of First Response® Combo RDT to diagnose peripheral malaria in pregnancy

Interest in the ability of RDTs to accurately detect malaria infections among persons in populations at risk of this infection has increased since tests started becoming commercially available well over a decade ago. This has stemmed largely from the limitations associated with the use of blood smear microscopy, which for decades had remained the main test available for malaria detection in most parts of the world where malaria is prevalent, and the ease of use of malaria rapid diagnostic tests,

often overcoming some of the difficulties associated with microscopy. Even though RDTs have shown promise as suitable replacement for blood smear microscopy, they have also come under intense scrutiny, to determine whether they are accurate enough in ordinary usage to allow them to be used in health facility laboratories in the management of cases of suspected malaria. Accordingly, many evaluations have been conducted of RDT performance to enable policy makers and programme managers to make appropriate choices among the increasing number of commercially available test kits.

Since 2008, the World Health Organisation (WHO), the Foundation for Innovative New Diagnostics (FIND), the United States Centers for Disease Control and Prevention (CDC) and other partners have conducted six rounds of testing of malaria RDTs. The rationale for this evaluation is based on the recognition by WHO that the use of antigen detecting rapid diagnostic tests is a major cornerstone of its recommendation that malaria case management be based on parasite diagnosis in all cases (WHO, 2015). Companies manufacturing RDTs according to ISO 13485:2003 were invited to submit one to three products for testing. The submitted products were tested against *P. falciparum* and *P. vivax* clinical samples diluted to 200 and 2,000 parasites/ microliter and with consistently comparable concentrations of HRP2, PLDH and aldolase determined by ELISA. In five rounds of testing, 210 products have been evaluated and 206 of these have progressed to testing against panels of patient derived parasites as well as a parasite-negative panel. Also thermal stability of the RDTs was assessed after two months of storage at elevated temperatures and humidity; a description of ease of use was also made. The evaluation determined that several RDTs consistently detected malaria at low parasite density (200 parasites/microlitre) in five rounds of testing, had low false-positive rates, were stable at tropical

temperatures, were relatively easy to use and can detect *P. falciparum* or *P. vivax* or both. Even though the performance of the products varied widely at low parasite density, all the products had a high rate of detection of *P. falciparum* at 2,000 or 5,000 parasites/microlitre. For *P. vivax*, majority of the products were able to detect parasites at 2,000 parasites/microlitre. The evaluation also showed that *P. falciparum* tests targeting the HRP2 antigen had the highest detection rates. The two poorest detecting tests for the detection of *P. falciparum* were based on *P. falciparum*-specific pLDH detection (WHO, 2014). In this evaluation the panel detection score (PDS) was used in rating the RDTs. The PDS is the percentage of malaria samples in the panel that give a positive result in two RDTs per lot at the lower parasite density or by a single RDT per lot at the higher density. In round 5 of testing, the First Response® Malaria Ag. pLDH/HRP2 Combo test used in this study, had a PDS of 85.0% and 74.3% for *P. falciparum* and *P. vivax* respectively at the lower parasite density (200 parasites/microlitre) and 100% for both *P. falciparum* and *P. vivax* at the higher parasite densities (2,000 or 5,000 parasites/microlitre). This RDT had 0.0% false-positive rate for *P. falciparum* at both low and high parasite densities and 0.3% false-positive rate for non-falciparum infection at low parasite density and 0.0% at high parasite density. From the laboratory evaluation therefore, the RDT performed very well in detecting *P. falciparum* and non-falciparum infections.

Ever since RDTs started becoming commercially available, they have been the subject of many trials in geographically diverse malaria-endemic regions all over the world. In many such areas, blood smear microscopy, which had remained the recommended diagnostic test for decades, was either unavailable or of poor quality owing to logistical reasons. Therefore RDTs have been tested with a view to determining if in addition to their ease of use, they were accurate enough to be used

in patient management. Also, WHO recommendation for universal parasitological diagnosis before treatment has meant that many countries have put in place protocols which require testing of suspected cases before treatment. These protocols for testing before treatment rest largely on rapid diagnostic testing. Accordingly, RDTs have been evaluated often to determine if they can serve as suitable screening tests. To meet this criterion, their sensitivity should be high enough to detect most infections and therefore have a low false-negative rate.

PCR, which was used as a reference test in this study, is known to have high sensitivity in the detection of malaria parasites with the ability to detect five parasites or less per microlitre of blood with 100% sensitivity and specificity (Snounou et al. 1993). In the last two decades several PCR-based methods have been developed for the detection of malaria (Barker et al. 1992; Jelinek et al. 1999; Kawamoto et al. 1996; Seesod et al. 1993; Sethabutr et al. 1992; Snounou et al.1993; Wataya et al. 1993; Wataya et al. 1991). Owing to their complexity, requirement of expensive equipment and labour-intensity PCR-based techniques have remained largely as sensitive standards against which other non-molecular methods are evaluated.

In this study, the sensitivity of the RDT to detect peripheral malaria parasitaemia in pregnancy using PCR as the reference ranged between 57.7% (95% CI 43.2-71.3) obtained at the delivery visit when the prevalence of malaria was 15.0% (95% CI 11.0-19.1) to 89.3% (95% CI 85.5-92.4) at the first study visit, when the prevalence of malaria was 57.0% (95% CI 53.0-61.0). The specificity of the RDT to detect malaria with PCR as the reference test ranged between 82.9% (95% CI 78.3-96.8) obtained at the third study visit to 88.2% (95% CI 83.6-91.9) at the first study visit. The RDT performed better as a screening tool at higher malaria prevalence as the sensitivity

declined with decreasing malaria prevalence. The specificity of the test was also highest during the first study visit when the malaria prevalence was highest.

When blood smear microscopy was used as the reference standard, the sensitivity of the RDT to detect peripheral malaria in pregnancy ranged between 60.3% (95% CI 46.6-73.0) obtained at the delivery visit when the prevalence of malaria by blood smear was 17.0% (95% CI 13.0-21.2) to 95.8% (95% CI 92.8-97.7) obtained at the first study visit when the prevalence of malaria by blood smear was 47.0% (43.0-50.9). Some studies have shown that even under the best conditions, blood slides tend to miss a good proportion of malaria in pregnancy cases when peripheral blood is examined (Leke et al, 1999). Some of these infections missed by blood slides may be picked up by PCR or RDT, accounting for the phenomenon of submicroscopic (subpatent) infections. Because PCR tends to pick up more infections and is therefore more sensitive than blood slide (Barker et al, 1992; Snounou et al, 1993), sensitivity of RDTs tends to be lower when PCR is used as the gold standard compared to when blood slide is used as the reference.

As presented in another section of this thesis, *P. falciparum* was the main species found among the pregnant women in this trial. In many of the evaluations of RDTs that have been done, the HRP2-based tests, which can only detect *P. falciparum,* were more sensitive in detecting this particular specie of parasite. Earlier laboratory trials of ParaSight F® RDT an HRP2-based test obtained average sensitivity of between 77 to 99% when the parasite density was >100 parasites/microlitre with specificity also ranging between 83 to 98% for *P. falciparum*, when compared with thick blood smear microscopy (Dietz et al. 1995; Kilian et al. 1997; Kodisinghe et al. 1997; Premji et al. 1994; Snounou et al. 1993). When the same RDT was compared to PCR, the sensitivity and specificity was 88% and 97% respectively (Humar et al. 1997; Pieroni et al.

1998). These RDT evaluations were performed in non-pregnant populations. When the First Response® Combo (pLDH/HRP2) RDT was compared with blood smear microscopy in a sample of 291 persons aged between 1-60 years reporting to clinics with febrile illness in the Jabalpur district in central India, an area where *P. falciparum* and *P. vivax* are prevalent, sensitivity of the RDT overall was 93% and specificity was 85%. Specifically for *P. falciparum*, the RDT fared better with a sensitivity of 96% and a specificity of 95% (Bharti et al. 2008). Another study in central India was conducted by Singh et al. (2010) among a total of 372 patients with a clinical suspicion of malaria, who were tested with several RDTs including the First Response® Combo (pLDH/HRP2) RDT. The analysis revealed that the First Response® Combo (pLDH/HRP2) was 94.7% (95% CI 89.5-97.7) sensitive and 69.9% (95% CI 63.6-75.6) specific for the detection of *P. falciparum* using blood smear microscopy as reference. For non-falciparum infections (*P. vivax*) the RDT was less sensitive [84.2% (95% CI 72.1-92.5)] but more specific [96.5% (95% CI 93.8-98.2)]. The sensitivity of the RDT to detect *P. falciparum* was less [83.6% (95% CI 77.2-88.8)] when PCR was used as the reference test.

A number of evaluations of RDTs have been conducted among pregnant women in various countries using different type of RDTs under different conditions with different outcomes. Tagbor et al. (2008) assessed the performance of OptiMAL® dipstick RDT in the diagnosis of malaria infection in pregnancy. The OptiMAL® RDT is a pLDH detecting test produced by DiaMed AG-Cressier, Switzerland under licence from Flow Inc., Portland, Oregon. In this evaluation, the performance of this RDT was compared to microscopy. A total of 6,370 OptiMAL® tests were conducted on 4,500 pregnant women attending antenatal clinics at Nkoranza in Ghana. The RDT obtained an overall sensitivity of 96.4% (95% CI 95.6-97.3) and a specificity of 85.5% (95% CI

83.9-87.1). The investigators were able to show that the diagnostic accuracy of the test declined with decreasing parasite density. For instance, the sensitivity of the test was only 57.1% (95% CI 49.8-64.5) when the parasite density was below 50 parasites/microlitre. VanderJagt et al. (2005) working at the Jos teaching Hospital in central Nigeria also compared the OptiMAL® RDT with microscopy for detecting malaria in pregnancy among 268 women attending antenatal clinics. Positive results from either microscopy or RDT were confirmed with PCR. Even though the investigators did not present the sensitivity and specificity of the RDT in their analyses, they concluded that the OptiMAL® RDT did not detect malaria in the blood on pregnant women with low parasite densities but they also thought the test would be useful for detecting placental malaria. Similarly, Singer et al. (2004) also conducted an evaluation of MAKROmed®, an HRP2-based RDT among pregnant women in Burkina Faso. They showed that the sensitivity of the RDT compared to peripheral blood smear microscopy was 96% with specificity of 67%. However, when PCR was used as the reference standard, the sensitivity of the RDT was 89% with specificity of 76%.

Even though the sensitivity of the RDT to detect peripheral malaria parasitaemia ranged between 89.3% when PCR was used as the reference standard and 95.8% when blood slide was the reference standard during the first visit when prevalence was highest, these fell slightly short of WHO recommendations that to be a useful diagnostic, RDTs must achieve 95% sensitivity (WHO, 2000).This is particularly important given the potentially severe consequences of undetected malaria in pregnant women and their babies which makes it imperative that any screening test for malaria should achieve very high sensitivity.

8.4 Influence of sub-microscopic and RDT negative malaria infections on the Outcome of Pregnancy

It is well established in literature that malaria in pregnancy has deleterious effects on both the mother and her developing foetus. In areas of stable and high malaria transmission, women in their first and second pregnancies are at a greater risk than multigravida women to suffer the consequences of malaria in pregnancy (Steketee et al. 1996). Malaria in pregnancy detected by microscopy has been shown not to be an adequate indicator of the extent of malaria infections in pregnancy. Desowitz and Alpers (1992) showed in a study in Papua New Guinea that peripheral parasitaemia was only present in 50% of women with proven placental malaria.

In pregnancy, this may be due to the fact that red blood cells (RBCs) are sequestered in the placenta and not necessarily present in peripheral blood. This phenomenon is due to the adherence of infected RBCs expressing variant surface antigens (VSA) which selectively bind to chondroitin sulphate A in intervillous syncitiotrophoblasts in the placenta (Fried & Duffy, 1996; Maubert et al. 1997; Reeder et al. 1999).

It is also known that low-density parasitaemia often cannot be detected by conventional light microscopy as its sensitivity is limited. In the last two decades however, the development of molecular techniques has improved the detection of malaria infection. Molecular detection techniques have much higher sensitivity for the detection of malaria (Snounou et al. 1993) and have revealed the widespread presence of infections with parasite densities below thresholds usually detected by microscopy or RDTs. Clinically these submicroscopic infections have been associated with adverse effects during pregnancy (Arango et al. 2010).

Submicroscopic malaria infections

In many studies of malaria in pregnancy where both microscopy and PCR-based detection methods were used, it has often been found that twice as many women were infected with malaria as detected by PCR than those detected by microscopy (Mockenhaupt et al. 2000; Schleiermacher et al., 2001). This indicates that a large number of women habour sub-microscopic malaria infections i.e. parasite DNA is detected in their blood but parasites are not seen in blood smears (Walker-Abbey et al. 2005).

In this study, the prevalence of sub-microscopic malaria infections ranged from 10.6% obtained at first visit of the women in the study, to 4.7% at the fourth visit. These estimates are relatively low when compared with the prevalence of microscopic malaria infection of 47.9% at first visit and 12.1% at the fourth visit respectively.

In an earlier study in the forested hills in the Ashanti region of Ghana, Mockenhaupt et al. (2000) found that among a sample of 530 pregnant women, 32% had malaria parasites detectable by microscopy. However, the prevalence of malaria increased to 63% when PCR was used in examining the blood samples of the same women indicating a sub-microscopic malaria prevalence of 31%. In a similar study in Senegal, Schleiermacher et al. (2001) in a study of pregnant women, obtained malaria prevalence by microscopy of 29%, increasing to 85% when PCR was used, giving a submicroscopic infection prevalence of 56%. In Cameroon, Walker-Abbey et al. (2005) in a study of 278 women found that 27.5% of them were blood smear positive while 54.9% of them had sub-microscopic infections. In these reported studies therefore, the proportion of women with sub-microscopic infections was either about the same or more than those with microscopic infections. In this study however, the proportion of women with sub-microscopic infections was

smaller than those who had microscopic infections. Also, the proportion of sub-microscopic infections declined as the prevalence of malaria declined with subsequent antenatal clinic visits.

When the prevalence of sub-microscopic infections was analysed by the background characteristics of the study women at various visits there was no clear trend or pattern to the prevalence by age group or educational status. There was also no clear difference between the women in the IPT group and those in the IST group. However, secundigravid women had higher prevalence of sub-microscopic infections compared to primigravid women, for each of the three visits analysed even though the confidence intervals overlapped. This may be due to the fact that by the second pregnancy, women already may have started developing some levels of immunity to malaria in pregnancy leading to lower parasite densities. This is in consonance with findings that immune mechanisms against malaria parasites are gradually acquired with successive pregnancies (Fried et al. 1998; Beeson et al. 1999).

Sub-RDT malaria infections

RDTs have become increasingly important in the early detection and management of malaria and their use is being promoted widely. WHO recommends that all persons of all ages in all epidemiological settings with suspected malaria should receive a parasitological confirmation of diagnosis by either microscopy or RDT. Owing to the difficulties associated with blood smear microscopy such as non-availability of electricity, lack of well-trained technicians to read slides and lack of logistics, the use of RDTs has become a more viable option for many in poor countries trying to try to achieve the WHO recommendation of universal testing for suspected malaria cases.

In this study, the prevalence of sub-RDT infections, defined as the proportion of women who were negative by RDT but had PCR positive for any malaria infection ranged between 6.3% for women

at delivery visit to 3.4% for women attending their second scheduled antenatal clinic visit. Comparing the proportions of sub-microscopic infections described in the previous section to the proportions of sub-RDT infections, it is obvious that fewer sub-RDT infections picked up by PCR but not by RDT were being missed at the antenatal clinic visits compared to the sub-microscopic malaria infections being missed by microscopy.

A possible reason for the higher proportion of missed infections at delivery may be the acquisition of some level of immunity as the pregnancy progressed resulting in a greater chance that infections around that time are more likely to be of lower density. Also, previous malaria treatment could also lower parasite densities as noted by Kyabayinze et al. (2016). Further, higher density infections would most likely have been picked up earlier by RDT and treated. It has to be noted though, that this study only enrolled primigravidae and secundigravidae and not multigravidae in whom mean parasite densities generally tend to be lower.

A major purpose of this analyses was to determine whether RDTs could adequately be used for Intermittent Screening and Treatment (IST) should such regimens be adopted to replace existing IPTp programmes. The concerns expressed against such a potential change in programmes relate to the proportions of infections missed by the RDT used and whether there are any potential adverse consequences from such missed infections. However, in our analysis, sub-patent malaria infections (both sub-RDT and sub-microscopic) infections were neither associated with a reduction in birth weight nor with low birth weight. Sub-patent infections were also not associated with anaemia in pregnancy. However, these findings could have been influenced by iron and folate supplements which were given to the study participants as is done routinely by protocol in the study countries. This adds to the evidence which suggests that sub-patent infections have no

significant adverse effects on pregnancy as found by Cohee et al. (2014) and Singh et al. (2015). However, it must be noted that others have found significant association between sub-patent malaria infections and low birth weight (Mankhambo, Kanjala, Rudman, Lema and Rogerson, 2002; Mohammed et al., 2013) while other have found an association between sub-patent malaria infections and maternal anaemia (Mockenhaupt et al., 2000; Adegnika et al., 2006 and Cottrell et al., 2015). The reasons for these different findings may be due to the fact that different types of RDT and PCR assays were used in the various studies. The differences may also be related to variations in the characteristics of the study populations and study sample sizes. Owing to conflicting findings from different studies, these findings need to be interpreted carefully, particularly so as the sample size for this analysis was not large and the study was not sufficiently powered to detect small effects of sub-patent malaria infections.

8.5 Prevalence of malaria infection in pregnancy in four West African countries

Non-falciparum malaria Infections

It was quite apparent from this study that the diagnosis of non-falciparum malaria is difficult in ordinary practice. Two microscopists independently read the blood smears in each country. Six non-falciparum malaria cases were detected by the first readers while six cases were also detected by the second readers. However, there was only one confirmed case which was detected by both readers. This was a case of *P. malariae* which was detected in a woman enrolled at the Burkina Faso site of the study.

The sensitivity of microscopy to detect non-falciparum malaria in our study was clearly very low when compared to PCR which detected 40 cases in this sub-sample of women. This finding underlies the potential for many non-falciparum malaria cases being missed in everyday practice

by microscopists in laboratories where microscopy remains the mainstay of malaria laboratory diagnosis and stresses the need for more sensitive but simple and cheap methods of malaria diagnosis to be developed.

The prevalence of non-falciparum malaria at first ANC attendance in this study as detected by PCR was 1.72% overall, with mono infections accounting for 0.48% while mixed infections were found among 1.24% of the women. Compared to the overall prevalence of *P. falciparum* infection of 38.39% the prevalence of non-falciparum malaria is very low among pregnant women in the four countries in the study. Even though this prevalence is low, it demonstrates that non-falciparum infections are present among pregnant women in West Africa as its prevalence ranged from 0.28% in The Gambia to 4.98% in Mali. The finding of Mali obtaining the highest prevalence of non-falciparum malaria was significant as that country had the second lowest prevalence of *P. falciparum* infection among the four countries in the trial. This suggests that the relatively high prevalence of non-falciparum malaria found in Mali compared to the other countries in the trial cannot be explained by differences in the transmission intensity alone and this finding may just indicate a greater presence of non-falciparum species in that country. Also, all the non-falciparum species tested for in this study were found in Mali, including *P.vivax. P. vivax* has been detected in the northern parts of Mali in a previous study (Bernabeu et al, 2012). However, these *P. vivax* infections in this study were detected in Malian women enrolled in the southern parts of the country where vivax infections had not previously been reported. As expected *P. vivax* infections were not detected in any of the other countries in the trial. However, with the exception of Gambia which only recorded one case each of mono infections *of P. ovale curtisi* and *P. ovale wallikeri*, the other

countries in the study all had cases of *P. malariae* and *P. ovale* (*curtisi and wallikeri*) either as mono infections or mixed with *P. falciparum.*

Significantly, most of the non-falciparum infections detected were mixed infections with *P. falciparum.* A similar finding of mixed malaria infections of falciparum and non-falciparum malaria had been found in an earlier study among pregnant women in Ghana (Mockenhaupt et al, 2000). In Cameroon, Walker-Abbey et al (2005) also found a higher prevalence of mixed malaria infections compared to mono infections of non-falciparum malaria. They found that the prevalence of *P. malariae and P. ovale* was 7.6% and 2.5% respectively compared to 9.4% who had mixed infections with more than one species.

The rather low prevalence of non-falciparum infections together with the higher incidence of mixed infections has potential implications for species detection by microscopy as the presence of *P. falciparum* parasites particularly when in present in high densities, may mask the presence of other species, making it difficult for even expert microscopists to detect them. This showed quite clearly in this study where microscopy only detected very few non-falciparum malaria cases.

Non-falciparum malaria infections not detected by RDT

Intermittent screening and treatment for malaria in pregnancy (IST) depends to a great extent on the ability of the RDT that is used to detect as many of the malaria infections among the pregnant women as possible to enable them receive effective treatment with current Artemisinin-Combination Therapy. Therefore a test with a high sensitivity is desirable. Even though other aspects of this thesis deal with the sensitivity and specificity of the RDT used in the study to screen women for malaria, the analysis in this section dwelt on the ability of the RDT to detect non-falciparum malaria among the women who were screened. The RDT detected only three out of

nine mono non-falciparum infections. Given that this RDT is a combo test and should therefore detect falciparum and non-falciparum species, this finding fell below expectation. The test however appeared to have performed better at detecting the mixed infections, giving positive results for all the mixed infections except two cases out of nine cases of *P. falciparum* mixed with *P. malariae* that were reported negative. Unfortunately, the test results for the RDT did not indicate which bands were present at the time of a positive test and therefore it is not possible to differentiate a positive test with two bands from a positive test with only one band. When two test bands are positive (both HRP2 and pLDH) it may indicate *P. falciparum* alone or in combination with non-falciparum malaria as the *P. falciparum* parasite has both HRP2 and pLDH proteins. Differentiation is only possible when only the pLDH band is positive as this indicates that the parasite detected is a non-falciparum species as any *P. falciparum* present should have resulted in a positive HRP2 band as well. Therefore, it is probable that the test was detecting most of the mixed infections because *P. falciparum* was present, as it failed to perform equally well when detecting non-falciparum mono infections.

Risk Factors for non-falciparum malaria

The country of residence of the woman and the season of enrollment were the only factors which had a significant association with having a non-falciparum malaria infection. As discussed in an earlier section, women in Mali had a higher prevalence of non-falciparum malaria. However, owing to the small numbers of non-falciparum infections the estimates were not very precise, with large confidence intervals resulting. Women enrolled in Ghana were also more likely to have non-falciparum malaria when compared to Gambian women.

Season of enrollment is associated with malaria transmission in general. However, we also found that it is associated with a greater risk of non-falciparum malaria as women enrolled in the late wet season had a greater risk of non-falciparum malaria.

Prevalence of malaria in pregnancy detected at enrolment

The prevalence of any species of malaria obtained by microscopy among the women in the study ranged from 8.4% (95% CI 6.5-10.7) for the women enrolled in the Gambia to 19.6% (95% CI 16.2-23.5) for the Malian women, to 42.9% (95% CI 38.5-47.4) for the women enrolled in Burkina Faso. The highest prevalence of 48.9% (95% CI 45.2-52.7) was obtained among the women enrolled at the Ghana site. The prevalence of malaria by PCR was higher than that obtained by microscopy in all the four countries in the trial; in the Gambia it was 13.7% (95% CI 11.3-16.4), in Mali the prevalence was 31.3% (95% CI 27.2-35.6), in Burkina Faso it was 60.1% (95% CI 55.6-64.5) and in Ghana the prevalence by PCR was 60.3% (95% CI 56.6-63.9).

This finding is consistent with findings from other studies where parasitaemia was detected by both microscopy and PCR (Mockenhaupt et al. 2000, 2006; Schleiermacher et al. 2001; Mankhambo et al. 2002; Walker-Abbey et al. 2005; Malhotra et al. 2005; VanderJagt et al. 2005). However, findings by some authors (Mockenhaupt et al. 2000; Adam et al. 2005; Malhotra et al. 2005; Walker-Abbey et al. 2005;Adegnika et al. 2006) suggesting that PCR-based detection methods detect at least twice as many pregnant women infected with malaria compared to microscopy was not established in this study in any of the four countries even though PCR always detected a higher proportion of infections than microscopy. There is clear evidence that malaria prevalence has been decreasing in some African countries in the last decade (Bhattarai et al. 2007;

Ceesay et al. 2008, 2010; Bouyou-Akotet et al. 2008; Graves et al. 2008; O'Meara et al. 2008; Otten et al. 2009; Teklehaimanot et al. 2009; Lee et al. 2010; Beiersmann et al. 2011). The decline of malaria prevalence in the Gambia for instance, has been documented (Ceesay et al. 2010). These declines have been attributed to the scaling up of such interventions as artemisinin-based combination therapy (ACT), long lasting insecticide treated bed nets, intermittent preventive treatment in pregnancy (IPTp) and lately seasonal malaria chemoprevention (SMC) [WHO, 2012]. SMC, which was previously known as Intermittent Preventive Treatment in children (IPTc), is defined as:

"The intermittent administration of full treatment courses of an antimalarial medicine during the malaria season to prevent malarial illness with the objective of maintaining drug concentrations in the blood throughout the period of greatest malarial risk".

SMC is targeted at preventing most of the childhood malaria mortality and morbidity which occurs during a short rainy season in the Sahel sub-region.

This phenomenon of declining malaria prevalence, has not been observed in all the countries involved in the trial. In Mali, Coulibaly et al. (2014) followed up a cohort of 400 children aged 0 to 14 years for four years to document malaria incidence using cross-sectional surveys to measure malaria and anaemia prevalence; they concluded that despite the scaling up of malaria interventions, malaria incidence did not decrease in their study area. In Burkina Faso, Ramroth et al. (2009) also showed that malaria prevalence had not declined. They had found that even though all-cause mortality in children had declined over a period, malaria-specific mortality among the deaths of children studied had remained fairly stable.

This study found that malaria prevalence was higher at first ANC visit than subsequent visits. In Ghana and Burkina Faso, the prevalence was very high among these primi- and secundi-gravid women whether using microscopy or PCR assays at enrolment. The prevalence was quite moderate in Mali and relatively low in the Gambia when compared to Ghana and Burkina Faso. The reasons for these differences among these countries is not very obvious. However, the various country situations are a reflection of the funding available to the country malaria control programmes and how they are being implemented to eliminate malaria. As part of this trial, parasites obtained from study women at enrolment were genotyped for markers of SP resistance and the K540E mutation which predicts dihydrofolate reductase-dihydropteroase synthetase (dhfr-dhps) quintuple haplotype, which indicates a high risk of SP failure (Naidoo and Roper, 2010) was not found in Burkina Faso, Ghana and Gambia (Tagbor et al. 2015). In Mali, it was only found at a low frequency (<1%) in one of the sites but not at the other site. A parallel study to this trial on the in-vivo sensitivity of SP which was conducted at two of the study sites (Burkina Faso and Mali) showed that SP was still highly efficacious in asymptomatic pregnant women attending ANC (Coulibaly et al. 2014). This suggests that SP is still likely to remain efficacious for use in IPTp in these countries particularly in Ghana and Burkina Faso where prevalence of malaria among pregnant women at enrolment is so high. There is the need to ensure that coverage of IPTp is high in such areas. Also frequent dosing may prolong the protective effect of IPTp-SP (White, 2005; Kayentao et al. 2013). The World Health Organisation (WHO) Evidence Review Group on IPTp with SP has recommended the administration of SP to all pregnant women at each scheduled ANC visit starting in the second trimester and given one month apart, with the last dose given in the last trimester after 36 weeks (WHO, 2013). A total of five doses is thus envisaged. As the level of transmission below which IPTp is no longer useful is not known and consequently countries may

be reluctant to stop IPTp in low transmission settings without a non-inferior alternative, intermittent screening and treatment of malaria in pregnancy (ISTp) is an alternative which could be considered by policy makers and programme managers as this trial determined that it was non-inferior to IPTp-SP in preventing low birth weight, maternal anaemia and placental malaria (Tagbor et al. 2015).

8.6 Use of Symptoms to Predict Malaria in Pregnancy

In many malaria endemic areas, the diagnosis of malaria in children and adults has often been based on clinical symptoms and signs. However, the usual symptoms and signs that patients present with are not specific to malaria as they may also suggest other bacterial, viral or other diseases which are often prevalent in areas where malaria tends to be endemic. Clinical diagnosis of malaria can therefore be difficult because there is no component of the clinical features which is pathognomonic of the infection (WHO, 2000b) as all the features could suggest other tropical diseases or even urinary tract infections, which are known to be common in pregnancy.

The presentation of malaria in pregnancy may depend on such factors as the age of the woman, her gravidity, gestational age and her HIV status (WHO, 2004). The clinical manifestation of malaria in pregnant women is determined to a large extent by her level of acquired immunity which is also determined by the local malaria transmission characteristics. In areas of low unstable transmission, women often do not have sufficient acquired immunity and therefore malaria infection tends to be symptomatic, affects all parities and is associated with detectable parasitaemia (Brabin, 1983, 1985; Shulman et al. 2001; Adam et al. 2005). The main dangers of malaria in non-immune women include hyperpyrexia, hypoglycaemia, severe haemolytic anaemia, cerebral malaria and pulmonary oedema (Shulman and Dorman, 2000). However, in areas of intense stable

malaria transmission, malaria in pregnancy may be asymptomatic even though parasites tend to sequestrate in the intervillous bed of the placenta leading to maternal anaemia and low birth weight, particularly in paucigravidae (Brabin, 1983, 1985; Verhoeff et al. 1999; Shulman et al. 2001; Steketee et al. 2001). In a study in Malawi, Mc.Dermott et al. (1988) observed that none of the 19 women who had parasitaemia at enrolment into the study had a history of fever or symptoms suggestive of malaria. Huynh et al. (2011) followed up a prospective cohort of women for two years in Benin, an area of stable malaria transmission and analysed the presence of symptoms suggestive of malaria infection in 982 women during antenatal clinic visits. The investigators concluded that the majority of the women were asymptomatic during their routine visits when infected with malaria. They found however, that fever was associated with malaria infection only when the women came for unscheduled visits.

However, Tagbor et al. (2008) concluded after studying pregnant women enrolled in a randomized clinical trial to determine the efficacy, safety and tolerability of chloroquine, amodiaquine (AQ), sulfadoxine-pyrimethamine (SP) and SP + AQ for the treatment of MiP in a stable transmission area in Ghana, that MiP may often be more symptomatic than previously documented and even though the symptoms may be mild and non-specific, they can still predict parasitaemia.

Against this background, the main objective of this analysis was to assess if a symptom score could be useful in determining clients at busy antenatal clinics who necessarily have to be tested with an RDT for malaria because of symptoms they present with. Ideally in a system where intermittent screening and testing has been instituted as a regime for malaria detection and management, all women presenting at antenatal clinics need to be tested with an RDT when they attend scheduled antenatal clinic visits. However, given constraints in busy clinics in developing countries, there

may be instances where owing to lack of some resources, not every woman can be tested at all visits. In such situations, symptoms may help to prioritise who has to benefit from limited resources.

In this study, 14.4% of the women reported being sick at their first visit while 13.0% reported being sick at the second visit. Only 5.1% and 3.8% of the women reported having a fever at the first and second visits respectively. The commonest symptom reported was headache, which was complained of by 17.6% of women at first visit and 12.1% at second visit. While it is known that many women may be prompted to attend their first antenatal clinic visit for a particular pregnancy because they may not be feeling too well, many women may not necessarily be feeling unwell and may just have elected to attend clinic on that day. This may account for the rather low proportions of women presenting with the symptoms on the checklist. However, of those not presenting with particular symptoms, very high proportions tested positive by RDT even though the proportions of those having those particular symptoms and testing positive by RDT was always slightly higher. It appears therefore that the symptoms were of low sensitivity and specificity to detect women with malaria infections.

Two symptom scores were created with symptoms reported at the first two study visits which were significantly associated with positive RDT. The four symptoms which were significant at first visit (fever, chills, headache and waist pain) were also significant at the second visit. Other symptoms significant at the second visit which were not significant at the first visit were: being currently sick, general weakness, dizziness, vomiting and painful urination.

For score 1, created from the first visit symptoms, there was a linear trend showing increasing proportions of RDT positive with increasing number of symptoms in the score. Score 2 also shows

a significant association with RDT positivity. However, the trend is not linear. In multivariate logistic regression analysis, for score 1, women with 3 or 4 symptoms were 3.87 times (95% CI 1.07-14.09, P=0.040) more likely to have a positive RDT compared to women who had no symptoms. For score 2, women with 3 to 8 symptoms were 3.43 times (95% CI 1.57-7.45, P=0.002) more likely to have a positive RDT compared to women without symptoms. These findings were obtained after controlling for age group, educational status, gravidity, socio-economic status and season of enrolment. Arguably, these findings suggest that one or two symptoms may not be enough to predict the presence of malaria. However, the presence of three or more of these symptoms listed earlier is a fairly strong indication of malaria infection and therefore the need for testing to confirm the suspicion. The accuracy of these scores is not sufficient indication to start treatment for malaria. However, as a screening tool to decide on testing for malaria, it should be of some help to health workers if decisions have to be made about who should benefit from limited RDT testing resources.

The sensitivity of the symptom scores were calculated using two scenarios for each score. The scenarios for the first score dichotomised it into “No symptom” versus “any symptom” on one hand and “No symptom or one or two symptoms” versus “3 or 4 symptoms” on another hand. The first scenario had a sensitivity of 24.7% while the second scenario had a sensitivity of 4.5%. For the second score, the first scenario compared “No symptom” versus “Any symptom” on one hand and “No symptom or 1 or 2 symptoms” versus “3 to 8 symptoms” on another hand. For the second score, the first scenario obtained a sensitivity of 32.3% while the second scenario yielded a sensitivity of 15.2%. Therefore, the sensitivities of the various scenarios created ranged from a low of 4.4% to 32.5%. This suggests that at best the symptom scores created would be good enough

to pick up only a third of pregnant women attending ANC who had a positive RDT. On their own therefore, the symptoms do not provide enough precision to predict malaria infection in the women.

A major limitation of this analysis is the sample size. The calculated sample size was 1,281 women. However, the women in the IST arm of the study, who were tested intermittently with RDTs were 653. This therefore reduced the power of this analysis.

8.7 Research Implications of study

Even though the First response® Combo RDT had a good profile in in-vitro testing in five rounds by the WHO/FIND/CDC and other partners, its failure to detect significant numbers of malaria infections gives cause for concern as many countries strive to achieve the WHO recommendation of universal testing of all suspected malaria cases. Also with intermittent screening and treatment (IST) of malaria in pregnancy increasingly emerging as an alternative to IPTp, particularly in areas where malaria prevalence has declined and/or SP resistance has reached levels where the drug is no longer useful in malaria prevention, there is the need to ensure that the true malaria cases that will go undetected by RDT testing will be reduced to the barest minimum. Accordingly, there is the need to develop more sensitive assays which will detect the presence of placental malaria and to conduct further research comparing in-vivo performance of sensitive RDTs to determine those better suited for screening pregnant women.

Findings from this study indicate that non-falciparum malaria even though present, are still largely uncommon among the population of women that we studied and *P. falciparum* remains the dominant species responsible for a great majority of malaria infections among pregnant women. Owing to the low prevalence of non-falciparum malaria among the women in our study, it was not

possible to look for their potential association with adverse pregnancy outcomes. Perhaps, a larger study may provide an opportunity to examine the effects of non-falciparum malaria on pregnancy in view of the fact that there largely is paucity of data in the literature on this subject even though there is scanty information on the effects of *P. vivax* infection on pregnancy.

Also, given that this study did not find any adverse effects of sub-RDT MiP on pregnancy outcomes which agrees with findings from some studies but is at odds with others, there is the need for further evaluation of the effects of sub-RDT malaria infections on pregnancy.

Our symptoms scores were not sensitive enough to predict women who had positive RDT results. There may be the need to develop better symptom-based scores which could serve as a useful pre-screening tool where IST is introduced.

8.8. Implications for policy and practice

Even though some malaria infections were undetected by the RDT that was used in the trial, our analyses showed that women who had RDT negative/PCR positive (sub-RDT) malaria infections were not at an increased risk of adverse pregnancy outcome when compared with women who had no malaria. This suggests that even though some malaria infections will be missed by RDTs when women are screened and treated at scheduled antenatal clinic visits, women with those infections may not suffer any deleterious effects from those infections possibly because those infections are of low density parasitaemia and hence are not detected by the RDT. Therefore screening with RDTs for malaria at antenatal clinics is a useful test to conduct for the control of malaria in pregnancy and should be instituted and maintained as the standard of care.

RDTs have become cheaper and increasingly available in the last decade or so. However, in many developing countries such as Ghana, supply of RDTs is not always guaranteed owing largely to

funding issues. Under these circumstances midwives at the antenatal clinics may have to use some mechanisms to determine women who necessarily have to be tested for malaria even in situations where not every woman can be tested. Our analyses showed that symptoms presented by women who may have parasitaemia may not be sensitive enough to indicate the presence of malaria when used alone. Although combining a few symptoms into a score gave a higher probability of predicting the presence of malaria infection this approach was not sensitive enough to recommend not offering IPT to women who test RDT negative. One possibility is a combination of IST with IPTp; first screen women using a symptom score to identify who should be tested with RDT. Give IPTp-SP to those women who are deemed to be asymptomatic and also to women who are RDT negative; and treat with an ACT if the RDT is positive. This approach to using symptoms to suggest which women necessarily need to be tested for malaria will also save some cost as well as ease pressure on scarce human resource which is often constrained in many developing countries. This will require midwives to actively solicit these symptoms from pregnant women attending antenatal clinics preferably using a checklist of symptoms most likely to suggest a positive RDT. Midwives will have to be trained to look out for these symptoms and ensure that women presenting with those get tested with an RDT even if they are receiving IPTp. In spite of these suggestions, it must be emphasized that restricting RDT testing to women who may need it based on symptoms which are often non-specific, is not ideal.

This PhD sub-study was also done with the objective of providing further evidence to support IST as an alternative to IPTp particularly in situations where SP is failing as a result of high-grade resistance. Even though this study provides some of that evidence by the finding that sub-patent malaria infections in this study were not associated with adverse outcomes such as low birth weight

or anaemia in pregnancy and therefore a few missed infections did not have negative consequences, other factors will have to be considered in transferring these results into policy. For instance, a cost effectiveness study was conducted for the main trial of IST versus IPTp which showed clearly that IST was less cost effective when compared to IPTp in these study countries where SP was still efficacious (Fernandes et al. 2016). However, simulations conducted in that study also showed that the cost-effectiveness of IST with Artemether Lumefantrine increased as the efficacy of SP decreased even though the specific point at which IST becomes cost-effective depends on other factors such as bed net use and coverage.

8.9 Conclusions

We also found that compared to PCR as the gold standard, the sensitivity and specificity of the RDT we used in our study was generally quite good. *P. falciparum* remains the predominant malaria species infecting pregnant women in the four study sites. The risk of malaria in pregnancy due to the other malaria species infecting humans (except *P. knowlesi*, for which we did not test), is very low. Even though RDTs will not detect all malaria infections, most probably infections with low density parasitaemia, women who may get missed by RDTs are not at great risk of developing adverse pregnancy outcomes when compared with women who had no malaria. A clinical symptoms based approach to screen women requiring RDT was not sensitive enough to be recommended. All pregnant women should be screened with RDT if IST was considered to replace IPTp-SP in areas where SP resistance is too high or transmission intensity is very low.

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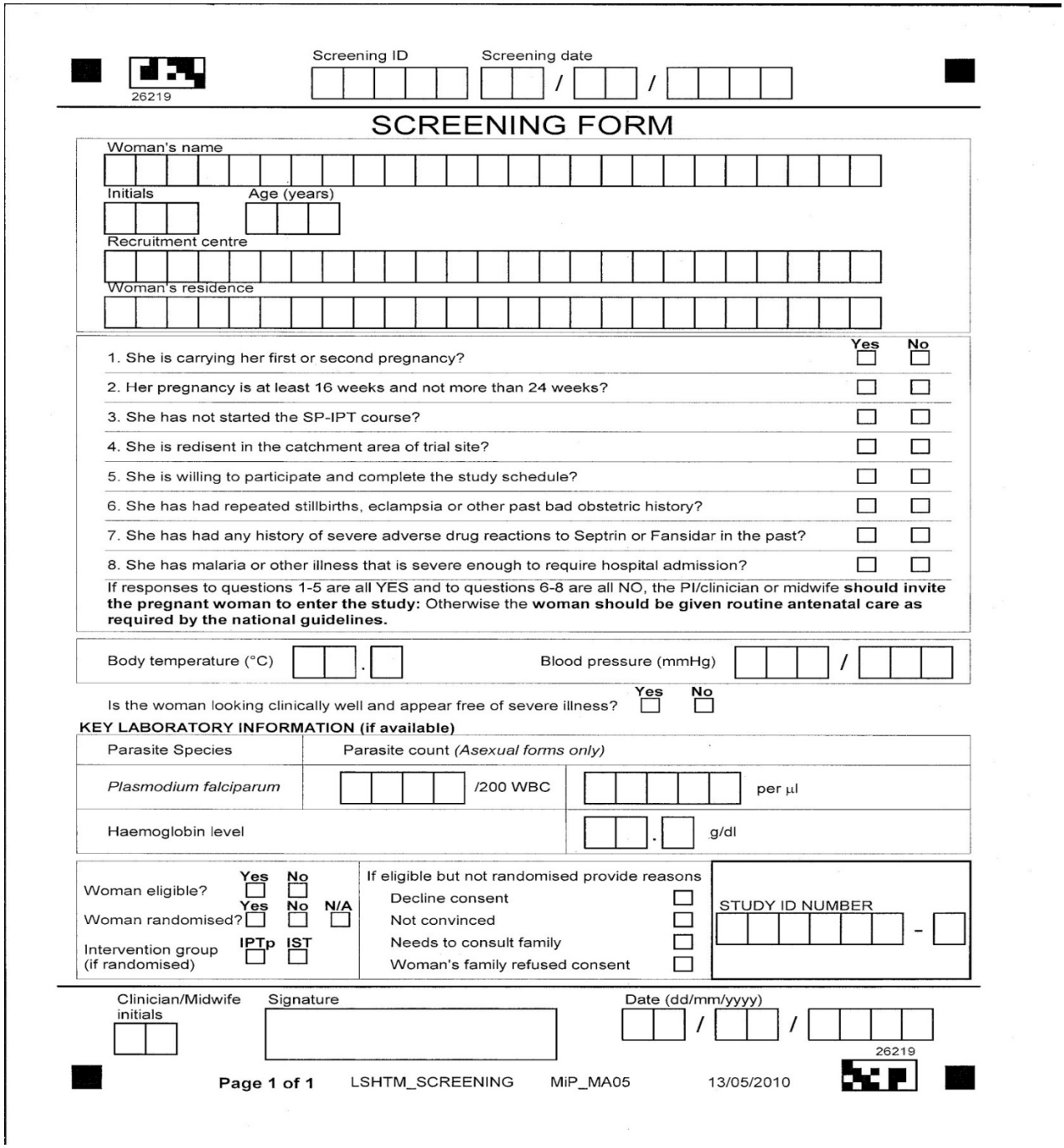
APPENDIX 1

Data Collection Forms

10.1 Screening Form



26219

Screening ID ☐☐☐☐☐ Screening date ☐☐/☐☐/☐☐☐☐

SCREENING FORM

Woman's name

Initials ☐☐☐ Age (years) ☐☐☐

Recruitment centre

Woman's residence

	Yes	No
1. She is carrying her first or second pregnancy?	☐	☐
2. Her pregnancy is at least 16 weeks and not more than 24 weeks?	☐	☐
3. She has not started the SP-IPT course?	☐	☐
4. She is redisent in the catchment area of trial site?	☐	☐
5. She is willing to participate and complete the study schedule?	☐	☐
6. She has had repeated stillbirths, eclampsia or other past bad obstetric history?	☐	☐
7. She has had any history of severe adverse drug reactions to Septrin or Fansidar in the past?	☐	☐
8. She has malaria or other illness that is severe enough to require hospital admission?	☐	☐

If responses to questions 1-5 are all YES and to questions 6-8 are all NO, the PI/clinician or midwife **should invite the pregnant woman to enter the study:** Otherwise the **woman should be given routine antenatal care as required by the national guidelines.**

Body temperature (°C) ☐☐.☐ Blood pressure (mmHg) ☐☐☐/☐☐☐

Is the woman looking clinically well and appear free of severe illness? Yes ☐ No ☐

KEY LABORATORY INFORMATION (if available)

Parasite Species	Parasite count *(Asexual forms only)*	
Plasmodium falciparum	☐☐☐☐ /200 WBC	☐☐☐☐☐ per μl
Haemoglobin level		☐☐.☐ g/dl

Woman eligible? Yes ☐ No ☐

Woman randomised? Yes ☐ No ☐ N/A ☐

Intervention group (if randomised) IPTp ☐ IST ☐

If eligible but not randomised provide reasons

- Decline consent ☐
- Not convinced ☐
- Needs to consult family ☐
- Woman's family refused consent ☐

STUDY ID NUMBER ☐☐☐☐☐☐ - ☐

Clinician/Midwife initials ☐☐ Signature ☐ Date (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

26219

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10.2 Demography Form

13523 Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date ☐☐ / ☐☐ / ☐☐☐☐

DEMOGRAPHY FORM

Date of Birth (DD / MM / YYYY) ☐☐ / ☐☐ / ☐☐☐☐ Age ☐☐

Q1 Education level reached *(cross one)*
1. None ☐
2. Basic ☐
3. Secondary ☐
4. Tertiary ☐

Q2 Occupation *(cross one)*
1. Housewife ☐
2. Farmer ☐
3. Trader ☐
4. Salary worker ☐
5. Other ☐

Q3 Religion *(cross one)*
1. Christianity ☐
2. Islam ☐
3. Traditional African Religion ☐
4. None ☐
5. Other ☐

Q4 Marital status *(cross one)*
1. Married ☐
2. Single ☐
3. Divorced ☐
4. Separated ☐
5. Widowed ☐

Q5 What is the main source of drinking water in your household? *(cross one)*
1. Surface water ☐
2. Tanker ☐
3. Well ☐
4. Bore hole ☐
5. Piped water ☐
6. Other ☐

Q6 What kind of toilet facilities does your household use? *(cross one)*
1. No facility ☐
2. Pit latrine ☐
3. Improved pit latrine (KVIP) ☐
4. Flush toilet ☐
5. Other ☐

Q7 Does your household have?

	Yes	No
Electricity	☐	☐
Radio	☐	☐
Television	☐	☐
Fixed telephone	☐	☐
Freezer / refrigerator	☐	☐
Chicken / ducks / goats	☐	☐

Q8 What type of fuel does your household mainly use for cooking? *(cross one)*
1. Firewood ☐
2. Charcoal ☐
3. Gas ☐
4. Electricity ☐
5. Other ☐

Q9 What is the main material of the floor? *(cross one)*
1. Earth / sand ☐
2. Wooden / bamboo ☐
3. Vinyl / tiles ☐
4. Cement ☐
5. Other ☐

Q10 What is the main material of the roof? *(cross one)*
1. Plastic / tarpaulin ☐
2. Thatch ☐
3. Iron sheets or tiles ☐
4. Other ☐

Study ID

13523 [ ][ ][ ][ ][ ][ ] - [ ]

Q11 Does any member of your household own?

	Yes	No
Mobile phone	☐	☐
Bicycle	☐	☐
Motorcycle / scooter	☐	☐
Car / truck	☐	☐
Ox cart / horse cart	☐	☐

Q12 Does your household have any mosquito bed nets?

(cross one)

1. Yes ☐
2. No ☐
8. Don't know ☐

If NO, skip to Q19

Q13 How many mosquito bed nets does your house have?

[ ][ ]

Q14 Where did your household obtain your mosquito nets?

(cross all that apply)

1. Health facility ☐
2. Community programme / distribution ☐
3. Shop / pharmacy ☐
4. Market ☐
5. Gift ☐
6. Other ☐
8. Don't know ☐

Q15 If it was not free, how much did you pay for a net?

[ ][ ][ ][ ][ ]

Q16 When you got the mosquito net was it already treated?

(cross one)

1. Yes ☐
2. No ☐
8. Don't know ☐

Q17 Did you sleep under a treated bed net last night?

(cross one)

1. Yes ☐
2. No ☐

Q18 If NO, why?

(cross all that apply)

1. Reserved for my baby ☐
2. It is difficult to hang ☐
3. It feels hot ☐
4. I am afraid ☐
5. I forgot ☐
9. Not applicable ☐

Q19 What are the benefits of a mosquito net treated with insecticide?

(cross all that apply)

1. Protects against mosquito bites ☐
2. Kills mosquitos on contact ☐
3. Reduces the frequency of malaria attacks ☐
4. Reduces the risk of maternal anaemia ☐
5. Reduces the risk of low birth weight ☐
6. Reduces the risks of illness and death among children ☐
8. Don't know ☐

10.3 Medical and Obstetric Assessment Form

Study ID [ ][ ][ ][ ][ ][ ] - [ ] Initials [ ][ ][ ] Date [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

22328

MEDICAL AND OBSTETRIC ASSESSMENT FORM

SYMPTOM	Yes	No
1. Currently sick	☐	☐
2. Fever	☐	☐
3. Chills	☐	☐
4. Headache	☐	☐
5. Nausea	☐	☐
6. Vomiting	☐	☐
7. Diarrhoea	☐	☐
8. General weakness	☐	☐
9. Dizziness	☐	☐
10. Easily tired	☐	☐
11. General malaise	☐	☐
12. Lower abdominal pain	☐	☐
13. Painful urination	☐	☐
14. Waist pain	☐	☐
15. Cough	☐	☐
16. Other	☐	☐

10.4 Obstetric Assessment Form

Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date ☐☐ / ☐☐ / ☐☐☐☐

26162

OBSTETRIC ASSESSMENT FORM

1. Gravidity				☐☐
2. Parity				☐☐
3. Gestation				
a. History (Months)				☐☐ LMP ☐☐ / ☐☐ / ☐☐☐☐
b. Measured fundal height (cm)				☐☐
c. Ultrasound scan (weeks)				☐☐
4. No. of children alive				☐☐
5. No. of children dead				☐☐
	Yes	**No**	**N/A**	
6. Previous ANC bleeding	☐	☐	☐	
7. Previous ANC admissions	☐	☐	☐	
8. Previous abortions	☐	☐	☐	
9. Bled in current pregnancy	☐	☐	☐	
10. Admitted with current pregnancy	☐	☐	☐	

11. Your last delivery point	1. Hospital	☐	*(cross one)*
	2. Health centre/Clinic/Maternity home	☐	
	3. TBA	☐	
	4. Self/Relative at home	☐	
	9. N/A	☐	
12. Type of delivery	1. SVD	☐	*(cross one)*
	2. Vacuum assisted	☐	
	3. Episiotomy	☐	
	4. C/Section	☐	
	5. N/A	☐	
13. Foetal heart sound	1. Heard	☐	*(cross one)*
	2. Not heard	☐	
14. Presentation	1. Cephalic	☐	*(cross one)*
	2. Breech	☐	
	3. Transverse	☐	

Clinician/Midwife initials ☐☐ Signature _____ Date (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

26162

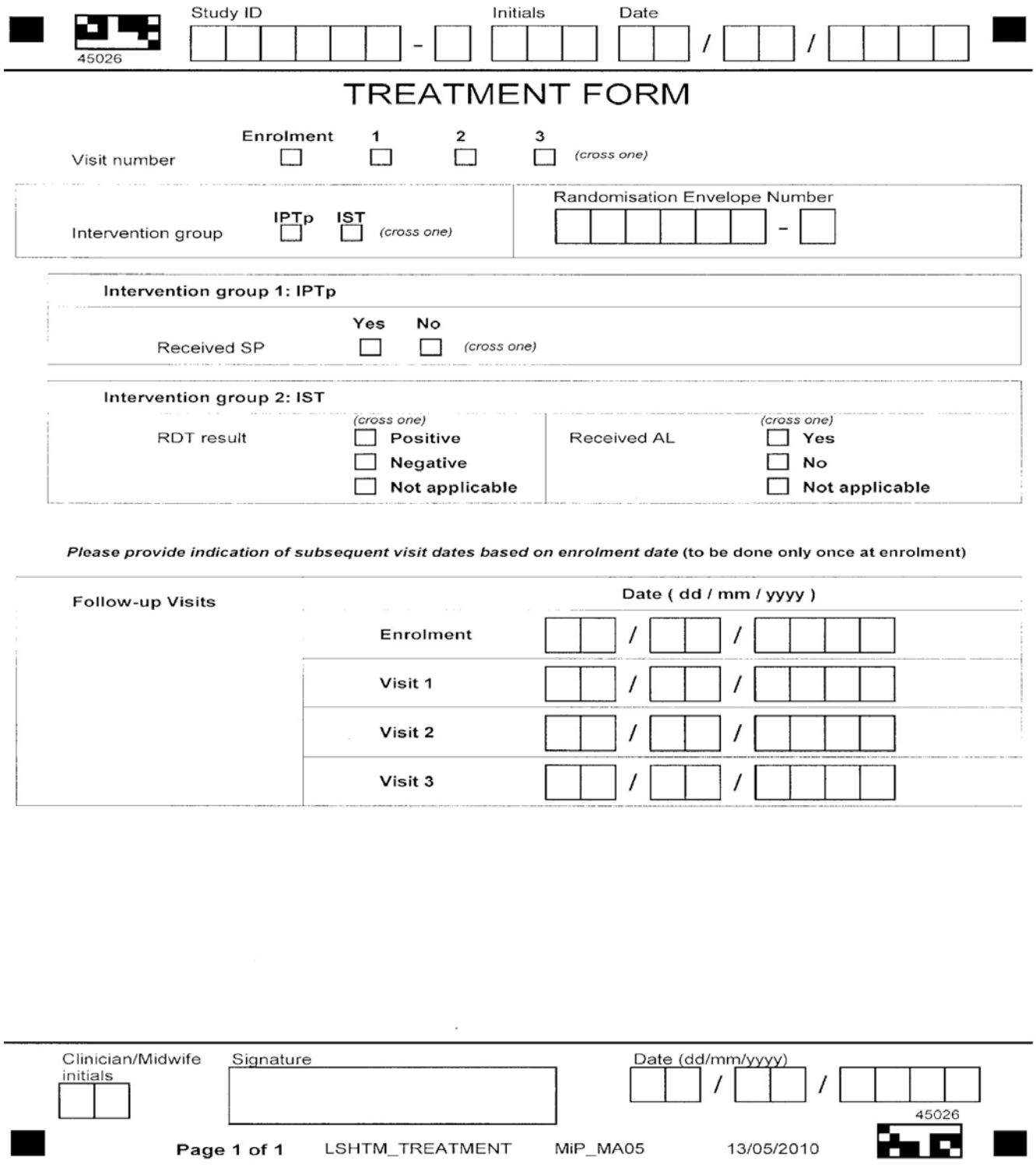
10.5 Physical Exam Form

Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date ☐☐ / ☐☐ / ☐☐☐☐

PHYSICAL EXAM

1. Body temperature (°C) (Axillary)	☐☐.☐
2. Pulse rate (Beats per minute via radial pulse)	☐☐☐
3. Blood pressure	☐☐☐ / ☐☐☐
4. Does the woman have pallor?	Yes ☐ No ☐
5. Which of the following areas of the body look pale?	Yes / No
a. Mucous membranes of the mouth	☐ ☐
b. Conjunctivae	☐ ☐
c. Palms	☐ ☐
d. Nail bed	☐ ☐
6. Does the woman have oedema of the feet?	Yes ☐ No ☐
7. Is the woman jaundiced?	Yes ☐ No ☐
8. Are the following organs listed below enlarged?	Yes / No
a. Liver	☐ ☐
b. Spleen	☐ ☐

10.6 Treatment Form



45026

Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date ☐☐ / ☐☐ / ☐☐☐☐

TREATMENT FORM

Visit number: Enrolment ☐ 1 ☐ 2 ☐ 3 ☐ *(cross one)*

Intervention group: IPTp ☐ IST ☐ *(cross one)*

Randomisation Envelope Number ☐☐☐☐☐☐ - ☐

Intervention group 1: IPTp

Received SP: Yes ☐ No ☐ *(cross one)*

Intervention group 2: IST

RDT result *(cross one)*: ☐ Positive ☐ Negative ☐ Not applicable

Received AL *(cross one)*: ☐ Yes ☐ No ☐ Not applicable

Please provide indication of subsequent visit dates based on enrolment date **(to be done only once at enrolment)**

Follow-up Visits	Date (dd / mm / yyyy)
Enrolment	☐☐ / ☐☐ / ☐☐☐☐
Visit 1	☐☐ / ☐☐ / ☐☐☐☐
Visit 2	☐☐ / ☐☐ / ☐☐☐☐
Visit 3	☐☐ / ☐☐ / ☐☐☐☐

Clinician/Midwife initials ☐☐ Signature ☐ Date (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

45026

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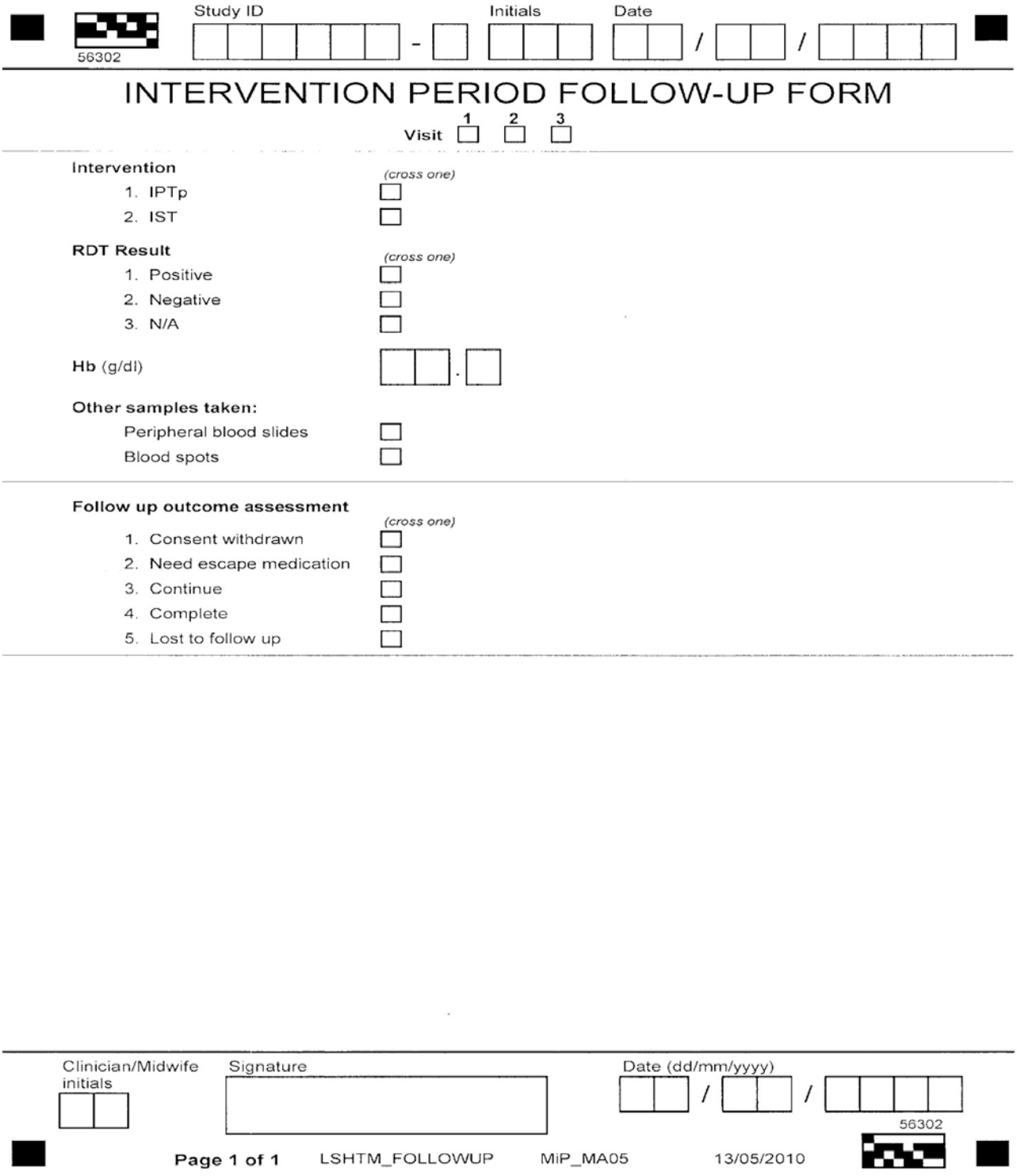
10.7 Intervention Period Follow-Up Form



Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date ☐☐ / ☐☐ / ☐☐☐☐

56302

INTERVENTION PERIOD FOLLOW-UP FORM

Visit 1 ☐ 2 ☐ 3 ☐

Intervention *(cross one)*

1. IPTp ☐
2. IST ☐

RDT Result *(cross one)*

1. Positive ☐
2. Negative ☐
3. N/A ☐

Hb (g/dl) ☐☐.☐

Other samples taken:

Peripheral blood slides ☐

Blood spots ☐

Follow up outcome assessment *(cross one)*

1. Consent withdrawn ☐
2. Need escape medication ☐
3. Continue ☐
4. Complete ☐
5. Lost to follow up ☐

Clinician/Midwife initials ☐☐ Signature ☐ Date (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

56302

10.8 Illness Complaint Form

59836

Study ID ______ - __ Initials ___ Date __ / __ / ____

ILLNESS COMPLAINT FORM

Please use this form to record signs and symptoms reported during unscheduled visits

CLINICAL DESCRIPTION OF COMPLAIN

Onset of complaint with respect to last treatment schedule *(cross one)*

1. Within 7 days after start of treatment ☐
2. Within 14 days after start of treatment ☐
3. Other ☐

List signs and symptoms only

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

ACTION TAKEN (about woman's condition)

(cross one)

1. Reassured ☐
2. Needed treatment on OPD bases ☐
3. Admitted ☐

ACTION TAKEN (about woman's treatment)

(cross one)

1. Rescue medication ***(if malaria diagnosed)*** ☐
2. Drug stopped ☐
3. Other medication given ☐

Hb (g/dl) __ . _

RDT Result

(cross one)

1. Positive ☐
2. Negative ☐

If an adverse event is suspected, the PI/CLINICIAN should please complete out adverse event form

Clinician/Midwife initials | Signature | Date (dd/mm/yyyy) __ / __ / ____

59836

Page 1 of 1 LSHTM_ILLNESS_COMPLAINT MiP_MA05 13/05/2010

Name of Health Unit | Study ID | Initials | Date

27471

BIRTH ASSESSMENT FORM

Delivery date / / Pre-delivery Hb . G/dl

Delivery Information

Pregnancy outcome *(cross one)*
1. Miscarriage (<28 wks) ☐
2. Preterm (28 - 35 wks) ☐
3. Term (>36wks) ☐

Mode of delivery *(cross one)*
1. Normal ☐
2. Assisted (vacuum, forceps, episiotomy) ☐
3. Caesarean Section ☐
4. Abortion ☐

Was the second stage prolonged? *(cross one)*
1. Yes ☐
2. No ☐

If yes, what was the reason for the prolonged labour? *(cross one)*
1. Obstructed labour / pelvis inadequate ☐
2. Breath birth / Multiple birth ☐
3. Mother weak ☐
4. Other: ☐

Estimated blood loss during labour or after delivery by woman in millilitres

Did she have difficulty in deliverying the placenta? *(cross one)*
1. Yes ☐
2. No ☐

First Baby

Sex *(cross one)*: Male ☐ Female ☐

Condition of the baby *(cross one)*: Alive ☐ Stillbirth ☐ Died soon after birth ☐

Birth weight: . kg

Head circumference: . cm

Length: . cm

Splenomegaly *(cross one)*: 1. Yes ☐ 2. No ☐

Hepatomegaly *(cross one)*: 1. Yes ☐ 2. No ☐

Apgar score (1-10): after 5 mins

Yellow eyes & body *(cross one)*: 1. Yes ☐ 2. No ☐

Malformations *(cross one)*: 1. Yes ☐ 2. No ☐

Specify malformation if any:

Samples taken: *(cross all that apply)*
Peripheral blood slide ☐
Placenta blood slide ☐
Placenta biopsy ☐

27471

Study ID ☐☐☐☐☐☐ - ☐

27471

Second Baby

Sex (cross one)	Condition of the baby (cross one)	Birth weight	Head circumference	Length
Male ☐ Female ☐	Alive ☐ Stillbirth ☐ Died soon after birth ☐	☐.☐☐ kg	☐☐.☐ cm	☐☐.☐ cm

Splenomegaly (cross one)	Hepatomegaly (cross one)	Apgar score (1-10)	Yellow eyes & body (cross one)
1. Yes ☐ 2. No ☐	1. Yes ☐ 2. No ☐	☐☐ after 5 mins	1. Yes ☐ 2. No ☐

Malformations (cross one) 1. Yes ☐ 2. No ☐

Specify malformation if any:

Third Baby

Sex (cross one)	Condition of the baby (cross one)	Birth weight	Head circumference	Length
Male ☐ Female ☐	Alive ☐ Stillbirth ☐ Died soon after birth ☐	☐.☐☐ kg	☐☐.☐ cm	☐☐.☐ cm

Splenomegaly (cross one)	Hepatomegaly (cross one)	Apgar score (1-10)	Yellow eyes & body (cross one)
1. Yes ☐ 2. No ☐	1. Yes ☐ 2. No ☐	☐☐ after 5 mins	1. Yes ☐ 2. No ☐

Malformations (cross one) 1. Yes ☐ 2. No ☐

Specify malformation if any:

27471

Ballard scoring for assessing gestational age at birth

Neuromuscular criteria *(Tick the best description of baby's appearance)*

	-1	0	1	2	3	4	5
posture *(cross one)*	NA	wrists arms hips & legs striaght ☐	wrists bent & legs slightly bent ☐	elbows hips & legs bent but do not reach 90 deg. ☐	elbows hips & legs bent to 90 deg. ☐	elbows & legs bent & drawn close to body ☐	NA
square window (wrist) *(cross one)*	>90 deg ☐	90 deg ☐	60 deg ☐	45 deg ☐	30 deg ☐	0 deg ☐	NA
arm recoil *(cross one)*	NA	180 deg ☐	140-180 deg ☐	110-140 deg ☐	90-110 deg ☐	< 90 deg ☐	NA
popliteal angle *(cross one)*	180 deg ☐	160 deg ☐	140 deg ☐	120 deg ☐	110 deg ☐	90 deg ☐	< 90 deg ☐
scarf sign *(cross one)*	elbow beyond opposite axillary line ☐	elbow to opposite axillary line ☐	elbow beyond opposite midclavicular line ☐	elbow to midline ☐	elbow to axillary line ☐	elbow does not reach axillary line ☐	NA
Heel to ear *(cross one)*	leg straight heel reaches ear ☐	leg straight heel reaches chin ☐	knee slightly bent heel reaches 140 deg from prone ☐	knee bent heel reaches 120 deg from prone ☐	knee bent to 90 deg heel reaches 90 deg from prone ☐	knee bent heel reaches 45 deg from prone ☐	NA

Study ID

27471

Physical criteria *(Tick the best description of the baby's appearance)*

	-1	0	1	2	3	4	5
skin *(cross one)*	sticky friable transparent ☐	gelatinous red trans-lucent ☐	smooth pink visible vains ☐	superficial peeling &/ or rash few viens ☐	cracking pale areas rare veins ☐	parchment deep cracking no vessels ☐	feathery cracked wrinkled ☐
lanugo *(cross one)*	none ☐	sparse ☐	abundant ☐	thinning ☐	bald areas ☐	mostly bald ☐	NA
breast *(cross one)*	imperceptible ☐	barely imperceptible ☐	flat areola no bud ☐	stippled areola 1mm bud ☐	raised areola 3 mm bud ☐	full areola 5 mm bud ☐	NA
genitals male *(cross one)*	scrotum flat smooth ☐	scrotum empty faint rugae ☐	testes in upper canal rare rugae ☐	testes descending few rugae ☐	testes down good rugae ☐	testes pendulous deep rugae ☐	NA
genitals female *(cross one)*	clitoris prominent labia flat ☐	clitoris prominent small labia minora ☐	clitoris prominent enlarging minora ☐	majora & minora equally prominent ☐	majora large minora small ☐	majora cover clitoris & minora ☐	NA

Total Ballard score: ☐☐ **Gestational Age (weeks):** ☐☐

Use the following table to convert Ballard scores to gestational ages in weeks. Round up Ballard scores to the highest score in the table e.g. scores ranging from 27-32 should be rounded up to 30 with corresponding gestational age as 36 weeks while scores ranging from 33-37 should be rounded up to 35 weeks with corresponding gestational age as 38 weeks

Correlation of scores with gestational age

Ballard score	-10	-5	0	5	10	15	20	25	30	35	40	45	50
Gestational Age in weeks	20	22	24	26	28	30	32	34	36	38	40	42	44

27471

Clinician/Midwife initials ☐☐ Signature ☐ Date (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

10.10 Placental History Form

3496 Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐ Date (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

Please tick the origin country for this sample

Site ID 2 4 (24=Gambia) Only The Gambia for this form

Study Country: Mali ☐ Burkina Faso ☐ Ghana ☐ Gambia ☐

PLACENTAL HISTOLOGY FORM

Evaluation Method, Sample Preservation and A Adequacy *(cross one)*

Q1. Evaluation, Conventional light	Yes ☐	No ☐
Q2. Evaluation. Polarized light	Yes ☐	No ☐
Q3. Hematoxylin and eosin stain	Yes ☐	No ☐
Q4. Giemsa's stain	Yes ☐	No ☐

	absent	mild	moderate	severe
Q5. Autolysis	☐	☐	☐	☐
	absent	mild	moderate	Abundant
Q6. Formalin pigment	☐	☐	☐	☐
	absent	mild	moderate	Abundant
Q7. Erythrocytes intervillous	☐	☐	☐	☐
	absent	Present		
Q8. Desidual basalis	☐	☐		
	absent	Present		
Q9. Amnios	☐	☐		

Parasites/Malarial Pigment

Q10. 500 maternal erythrocytes identified	Yes ☐	No ☐
Q11. If not, number of erythrocytes	☐	☐
Q12. Parasitized maternal erythrocytes	☐	☐
Q13. Percentage Parasitized maternal erythrocytes	☐	☐

Q14. Malaria pigment (excluding parasites):

- ☐ (1) Absent
- ☐ (2) Mild (identified only at 400X magnification)
- ☐ (3) Moderate (identified at 100X magnification but focally)
- ☐ (4) Abundant (identified at 100Xmagnification and wide spread)


3496

Study ID

Q15. Malaria pigment in free macrophages — Yes ☐ No ☐

Q16. Malaria pigment within fibrin — Yes ☐ No ☐

Q17. Parasitized fetal erythrocytes or pigment in villi — Yes ☐ No ☐

Q18. Intervillous inflammation: ☐ (1<5) ☐ (2=<5-10) ☐ (3=10-25) ☐ (4>25 per high power field)

Q19. Infracted areas: Absent ☐ Present ☐

Q20. Chorioamnionitis Absent ☐ Present ☐

Q21. Sample sent for confirmation? Yes ☐ No ☐

Please tick the country where this analysis or confirmation is done and add initials, signature and date

Analysis / Confirmation by:

☐ Gambia

☐ Spain (Barcelona)

Initials

Signature

Date (dd/mm/yyyy) / /

10.11 Parasitology Form

32227

GH 0 0 0 1 - 3 GA 2 3 / 1 2 / 2 0 1 1 23=Ghana, 24=Gambia

PARASITOLOGY FORM

Visit: Enrolment (0) ☐ 1 ☐ 2 ☐ 3 ☐ Delivery (4) ☐ Post-Partum(5) ☐ Unscheduled Visit ☐ Placental Smear ☐

SLIDE: ☐ Positive ☐ Negative

Species	Tick	Asexual forms		Sexual forms	
		Per 200 WBC	Per µl	Per 200 WBC	Per µl
1.P. falciparum	☐				
2.P. malariae	☐				
3. P. ovale	☐				
4. Pf + Pm	☐				
5. Pf + Po	☐				

READING: ☐1 ☐2

Confirmed by: Initials ☐☐ Signature ☐☐☐☐☐☐☐☐☐☐ Date (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

32227

Page 1 of 1 LSHTM_PARASITOLOGY_en_240611 MIP_MA05 09/09/2011

10.12 PCR – Molecular Analysis Form

6424

Study ID ☐☐☐☐☐☐ - ☐ Initials ☐☐☐

Please tick the origin country for this sample

Site ID 2 4 (24=Gambia) Only The Gambia for this form.

Study Country: Mali ☐ Burkina Faso ☐ Ghana ☐ Gambia ☐

PCR - MOLECULAR ANALYSIS FORM

Date Sample collected (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

Visit Enrolment (0) ☐ 1 ☐ 2 ☐ 3 ☐ Delivery (4) ☐ Post-Partum(5) ☐

PCR RESULT

	Yes	No
Plasmodium present?	☐	☐

Species	Tick
1. P. falciparum	☐
2. P. Malariae	☐
3. P. Ovale curtisi	☐
4. P. Ovale wallikeri	☐
5. P. Vivax	☐

	Yes	No
Sample sent for confirmation?	☐	☐

Please tick the country where this analysis or confirmation is done and add initials, signature and date

Analysis / Confirmation by:

☐ Giambia ☐ USA

Initials ☐☐☐ Signature ☐ Date (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐

APPENDIX 2

Ethics Approvals and Laboratory SOPs

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: **5545**

Name of Principal Investigator	**Brian Greenwood**
Department	**Infectious and Tropical Diseases**
Head of Department	**Professor Simon Croft**

Title: **A trial of intermittent preventive treatment with sulfadoxine-pyrimethamine versus intermittent screening and treatment malaria in pregnancy**

This application is approved by the Committee.

Chair of the Ethics Committee [redacted]

Date 22 July 2009

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

The Gambia Government / MRC Laboratories Joint
ETHICS COMMITTEE

c/o MRC Laboratories Fajara
P. O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4494498 or 4496 513
Tel: +220 – 4495442-6 ext. 2308

9 December 2009

Dr Kalifa Bojang
MRC Laboratories
Fajara

Dear Dr Bojang,

SCC 1167v3, 15 September 2009; Intermittent preventive treatment with sulphadoxine-pyrimethamine versus intermittent screening and treatment of malaria in pregnancy

Thank you for your letter dated 20 October 2009 providing the detailed and satisfactory responses to our Committee's queries regarding the above project. We note the inclusion of district and central members of NMCP as collaborators, the planned involvement of the PHC teams and the improved syntax and content of the Participant Information sheet. The project has this Committee's full approval

With best wishes

[redacted]
Mr. Malcolm Clarke
Chairman, Gambia Government/MRC Joint Ethics Committee

Additional documents submitted for review:

- Subject information sheet & Consent form, Version 2.0

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. :GHS-ERC: 3
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra

25th November 2009

Tel: +233-21-681109
Fax: +233-21-226739
Email: Hannah.Frimpong@hru-ghs.org

DR. ABRAHAM HODGSON, PRINCIPAL INVESTIGATOR

ETHICAL CLEARANCE

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"A Trial of Intermittent Preventive Treatment with Sulfadoxine-pyrimethamine versus intermittent screening and treatment of malaria in pregnancy" - ID NO: GHS-ERC-02/7/09

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

SIGNED.....[redacted]

PROFESSOR ALBERT GEORGE BAIDOE AMOAH
(GHS-ERC CHAIRMAN)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

In case of reply the number and date of this letter should be quoted.

My Ref.: appMIP01/09
Your Ref. No.

Navrongo Health Research Centr
Institutional Review Board
Ghana Health Service
P. O. Box 114
Navrongo, Ghana
Tel/Fax: +233-742-22348

irb@navrongo.mimcom.net

30th July, 2009

Dr. Abraham Hodgson
Navrongo Health Research Centre
Navrongo

ETHICS APPROVAL ID: NHRCIRB081

Dear Dr. Hodgson,

Approval of the protocol titled "*Intermittent preventive treatment with sulfadoxine-pyrimethamine versus intermittent screening and treatment of malaria in pregnancy*" *Dated: 11/10/2008*

Following your satisfactory address of the concerns raised by the NHRC IRB during its review of the above named protocol, the Board is pleased to grant you approval. The documents that were reviewed and approved included the study protocol, consent forms and data collection instruments all version 7 and dated 11th October 2008.

Please note that any amendment to these approved documents must receive prior NHRC IRB approval before implementation.

You are also to note that this approval expires on 29th July 2010. You are therefore required to submit a report for annual review two (2) months before the expiration date to renew your approval.

The Board demands that you report all unexpected serious adverse events within three (3) days verbally and within seven (7) days in writing.

The Board wishes you all the best in your study.

Sincerely,

Dr. John Awoonor-Williams
Chair, NHRC IRB

Cc: The Director, NHRC

MINISTERE DE L'ENSEIGNEMENT
SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE DE BAMAKO

FACULTE DE MEDECINE DE PHARMACIE ET
D'ODONTO-STOMATOLOGIE / BP 1805

☎ : (223) 20 22 52 77 🖷 : (223) 20 22 96 58

BAMAKO – MALI

N°09___84__/FMPOS

Le Président du Comité
D'Ethique de la FMPOS

Bamako, le 11 décembre 2009

/-)w **Docteur Kassoum KAYENTAO**
MD ; MSPH
MRTC/FMPOS

Cher Docteur,

J'ai le plaisir de vous informer que votre projet de recherche intitulé : **«Traitement préventif intermittent à la sulfadoxine-pyriméthamine (TPI – SP) versus dépistage et traitement du paludisme pendant la grossesse»** a été examiné par le Comité d'Ethique de la Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie de l'Université de Bamako au cours de sa séance du samedi 22 Août 2009 à 09 heures.

Le Comité d'Ethique a décidé de vous donner son accord pour l'exécution de vos travaux car vous avez satisfait aux conditions suivantes :

1. Revoir le titre en précisant les pays **(voir page du titre).**
2. Fournir la carte de l'Afrique avec les zones écoclimatiques des sites d'étude (les informations relatives à tous les sites sont données dans la page de description des sites au niveau de chapitre 6.2 de la méthodologie. L'établissement de la carte donnant les mêmes informations comme suggéré par le comité d'éthique nécessite un logiciel et une prestation des spécialistes demandant du temps et des couts). Nous adressons ce point à l'appréciation du comité d'éthique.
3. Ramener les éléments du budget au paragraphe budget en justifiant le budget affecté au personnel **(voir rubrique du budget dans les annexes)**.
4. Fournir l'avis des autres comités d'éthique **(voir approbation des 3 comités d'éthique du Burkina Faso, du Ghana et de Londres. Celle de la Gambie n'étant pas encore disponible).**

5. Préciser la prise en charge réelle du projet **(voir page 24 du protocole).**
6. Ajouter la liste des chercheurs maliens **(voir page de garde du protocole).**
7. Fournir les résultats au Comité d'Ethique de la FMPOS **(des rapports d'étape et définitif seront fournit au comité d'éthique de la FMPOS).**
8. Prévoir une visite de terrain de deux membres du comité d'éthique sur le site à la charge du Projet **(ceci a été prévu par le projet).**

Le Comité Institutionnel d'Ethique de la FMPOS vous souhaite plein succès.

LE PRESIDENT
DU COMITE D'ETHIQUE

[redacted]

Prof. Mamadou Marouf KEITA

MINISTERE DE LA SANTE

MINISTERE DES ENSEIGNEMENTS SECONDAIRE, SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

COMITE D'ETHIQUE POUR LA RECHERCHE EN SANTE

BURKINA FASO
Unité - Progrès - Justice

DELIBERATION N° 2009-41

1. TITRE DE LA RECHERCHE

Traitement préventif intermittent à la sulfadoxine-pyriméthamine (TPI-SP) versus dépistage et traitement du paludisme pendant la grossesse.

2. REFERENCE DU PROTOCOLE

Version du 29/06/2009

3. DOCUMENTATION

Protocole de recherche
Fiche d'information et de consentement des participants

4. REFERENCE DU DEMANDEUR

Investigateur principal : Dr S. O. Coulibaly
Co-investigateur principal : Dr B. Kouyaté

5. SITES DE LA RECHERCHE

- Gambie, Ghana et Mali,
- Burkina Faso : district sanitaire de Ziniaré,

6. DATE DE LA DELIBERATION

10 juillet 2009

7. ELEMENTS EXAMINES

- conception scientifique et conduite de la recherche ;
- soins et protection des participants à la recherche ;
- protection de la confidentialité des données du participant à la recherche ;
- processus de consentement éclairé.

8. OBSERVATIONS

- Préciser à la page 25 les dépenses prises en compte par le projet ;
- Inviter expressément, à la page 32, les femmes à se rendre systématiquement dans les centres pour toute affection en dehors des périodes de visites programmés pour bénéficier de la prise en charge ;
- Retirer du dernier paragraphe de la page 32, la 2e phrase intitulée « chaque traitement comporte des anti-malariques et des anti-anémiques » car contradictoire avec la première phrase ;
- Demander expressément, dès leur inclusion à l'étude, l'accord des participants pour une utilisation éventuelle des prélèvements pour d'autres recherches, si cela est prévu car il n'est pas évident de retrouver les patients plus tard pour requérir leur consentement ;
- Donner plus de précision sur les critères d'exclusion 4 et 5 de la page 19 (donner les critères de définition du SIDA clinique, préciser les principales maladies et les critères de diagnostic).

9. AVIS DU COMITE

Avis favorable

10. RESERVES

RAS

11. RECOMMANDATIONS

RAS

Ouagadougou, le 10 juillet 2009

Le rapporteur

[redacted]

Scholastique TRAORE

Le Président
P.I.

[redacted]

Dr. Ida Scholastique SAWADOGO

DNA EXTRACTION FROM WHOLE BLOOD, SOFT TISSUE OR FILTER PAPER BLOOD SPOTS USING THE QIA-XTRACTOR ROBOT		
Document type	Identification code	Implementation date
Standard operating procedure	SOP-MAL-DNA extraction from whole blood, soft tissue or filter paper blood spots using the QIA-Xtractor Robot	

	Name and job title	Signature*	Date*
Prepared by	Muna Affara Senior scientific officer, Malaria		
Reviewed by	Alieu Mendy, Scientific Officer, Malaria		
Approved by	Davis Nwakanma, Senior Manager Laboratory Services		

* To be hand-written to indicate approval

Version	Change history
Pilot	No change history- new document

Review date	Comments on review

Document**	Specific location
Master	Quality department
Electronic	Intranet
Confidential, unauthorised copying prohibited	

** The document owner can issue a controlled copy of this document to a responsible individual within a department. This individual will then be responsible for maintaining the controlled copy within their department on behalf of the document owner.

1. Abbreviations, contractions and definitions

MRC	Medical Research Council; representing MRC Unit, The Gambia
SOP	Standard operating procedure; work procedures and instructions implemented to meet the expectations of an internal policy, international or national guidelines.
The Unit	A term used to describe the MRC
GLP	Good Laboratory Practice
LB	Leadership Board
LM	Laboratory Management
WOI	Work Instructions

2. Background

To help meet standards of Good Laboratory Practice (GLP) and to make sure that all methods used in the research laboratory for the extraction of DNA are standardised.

3. Purpose

This standard operating procedure (SOP) addresses the procedure for the extraction of DNA from whole blood, soft tissue or filter paper blood spots using the QIA-Xtractor robot.

4. Scope

This SOP applies to all members of the MRC Unit, The Gambia research laboratories. This SOP can also be shared with other relevant research laboratories, where agreed and appropriate.

5. Responsibilities

5.1 The Leadership Board (LB) delegates the implementation of this SOP to Laboratory Management (LM).

5.2 LM ensures that appropriate health and safety precautions are applied to any relevant aspects of this SOP or associated work instructions (WOIs).

5.3 The laboratory supervisors are responsible for ensuring that the practices described in this procedure are complied with in their laboratories.

5.4 All members of the research laboratories are responsible for understanding and complying with the requirements of this SOP.

6. Prerequisites

6.1 Health and safety (H&S)

6.1.1 The procedure should be performed in accordance with the H&S practices described in SOP-H&S-001.
6.1.2 Refer to the laboratory H&S representative for any H&S advice.
6.1.3 It is important to wear gloves at all stages of the protocol, to avoid infection hazards from blood samples and to avoid contamination of samples with DNases/RNases from fingers.
6.1.4 Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste. Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids.
6.1.5 For any items contaminated with these buffers, clean with general laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.
6.1.6 Dispose of plasticware and liquid waste in accordance with laboratory guidelines for the sample type and reagent hazard.
6.1.7 Take care to avoid cross-contamination of samples and reagents.

6.2 Preparation and storage of reagents

6.2.1 PBS should be prepared fresh, autoclaved, and stored in the fridge.
6.2.2 Prepare and add reagents just before each run

6.3 Equipments, materials and reagents

6.3.1 ***The equipment, materials and reagents required to perform this procedure are:***

- One Low Skirt Glass Fibre Capture plate (Whatman 96 well low skirt 800µl GF/B Filter).
- One 2ml 96 Square Well Lysis Block.
- One 1.2ml 96-well Round-Well Sample Block.
- Two 200µl Sterile filter OnCor™C Tips in Robotic Rack.
- One Elution Plate with 0.65ml Cluster Tubes.
- Two disposable 70ml Reagent Tubs + Lids (re-usable).
- Two disposable 170ml Reagent Tubs + Lids (re-usable).
- One disposable 270ml Reagent Tub + Lid (re-usable).
- One Sheet of self adhesive PCR/Elisa plate Plastic Sealing Film (to seal unused wells of capture plate).
- Single-hole paper punchers.
- 2 empty 500ml beakers.
- Paper towel.
- 60ml of DX Binding (DXB) containing 0.6g of DX Binding Additive.
- 108ml of DX wash (DXW).
- 56ml of DX Final wash (DXF).
- 8ml of Elution Buffer or Molecular Biology grade Water.
- 21.7ml of DX Tissue Digest (DXT).
- 300µl of DX Digest Enzyme.
- DNA erase.
- Alpha-Q water.

7 Procedure

7.1 PRE-RUN DIGEST AND SAMPLE PREPARATION

7.1.1 If samples are frozen, thaw on ice and do a quick spin to collect samples to the bottom of the tubes.
7.1.2 Remove all reagents from the fridge and equilibrate to room temperature.
7.1.3 Prepare plate plan in a 96-well format with the sample IDs in your lab notebook.

NB: Pre-run digest does not apply to whole blood samples!!!
The capture plates' glass fibre membranes can be blocked by sample lysates with particulate matter or a high viscosity, or DNA overloading of the membrane.

7.1.4 Using a paper puncher, punch out 2-4 circles (3mm or 1/8 inch diameter punches) from each filter paper.
7.1.5 Place punched-out circles onto the bottom of each well of a Round-Well Sample block.
7.1.6 Between samples, dip paper puncher in DNA erase and blot dry on paper towel, then rinse by dipping in Alpha-Q water and blot dry. Repeat rinse in Alpha-Q water and blot dry on paper towel. **OR**
7.1.7 Aliquot 200ul of saliva sample into each well of the Round-Well Sample block, according to your plate plan. **OR**
7.1.8 Place ≈10mg of soft tissue or equivalent amount of 14ug of DNA onto the bottom of each well of a Round-Well Sample block.
7.1.9 Prepare your digest solution in a sterile 50ml falcon tube by adding 300µl of Digest Enzyme to 21.7ml of DXT and mix gently by inverting ten times.
7.1.10 Dispense 240µl of the above mix (digest solution) into each well of the 96 well Round-well Sample Block.
7.1.11 Ensure that the sample is completely immersed in the digest solution.
7.1.12 Incubate in an oven for 1hr at 60°C with intermittent vortexing every 15 minutes.
7.1.13 Centrifuge at 3000rcf for 5minutes and transfer 220µl of the supernatant to a clean Lysis block.

7.2 LOADING THE WORKSPACE PRIOR TO A RUN

7.2.1 Wipe down the X-tractor Gene™ workspace and Reagent Tub Lids using lint-free tissue paper or cloth in the following sequence: First spray with Milli-Q water and wipe dry, next spray with 1% Bleach solution and wipe dry, then spray again with Milli-Q water and wipe dry.
7.2.2 For whole blood, (if samples are frozen, thaw on ice and do a quick spin to collect samples to the bottom of the tubes) aliquot 50µl of sterile PBS into each well of the Lysis Block.
7.2.3 Add 150µl of whole blood sample to each well, mix by pipetting up and down 3X.
7.2.4 Observing sterile procedures, set up the instrument deck by loading the Capture Plate into position A1, the Elution Plate (unopened) to the right in the second vacuum sink and the Lysis Block into position B1 maintaining the same orientation for all plates in order to preserve Sample IDs.
7.2.5 Dispense reagent volumes indicated below (for a 96-well plate) using sterile 50ml or 15ml falcon tubes, as appropriate:

- 108ml of DX wash (DXW) into a 170ml tub Reagent tub and load in position A1 of the Reagent block R1.
- 56ml of DX Final wash (DXF) into a 170ml tub Reagent tub and load in position B1 of the Reagent block R1.
- 60ml of DX Binding (DXB) containing 0.6g of DX Binding Additive into the 270ml Reagent tub and load in Well 1 at position C1.
- Ensure that all the Reagents are covered with the appropriate lids.

7.3 STARTING A RUN

7.3.1 Start the control PC and click on the **'Corbett'** icon
7.3.2 Switch on the X-tractor Gene™ Robot.
7.3.3 Double click the **'Robotics 4'** icon on the Desktop.
7.3.4 Select **'Cancel'** on the vacuum extraction wizard window.
7.3.5 Click on **'File'**, **'Open'** and select the extraction protocol for your sample (**'My documents' 'MALARIA'** then choose the appropriate extraction protocol).
7.3.6 On the program window, click on '**wizard'** and select **'Vacuum DNA Extraction Wizard'**.
7.3.7 Select columns to extract from and click on **'Jump to End**'.
7.3.8 Review protocol and close the 'pop-up' window to return to Extraction protocol.
7.3.9 Select Sample wells at position B1 and enter sample IDs in the appropriate rows in the 'pop-up' window.
7.3.10 For whole blood, dispense 9ml of the Liquid Sample Digest Buffer (DXL) in a 15ml-falcon tube, add 1ml of DX Enzyme and mix gently by inverting 10X, taking care to avoid foaming. Decant into a 70ml Reagent tub and load onto Well2 at position C1 {½ life of this mixture is 10mins!!!}
7.3.11 Do not load Elution Buffer yet but keep at room temperature.
7.3.12 Load two full boxes of 200µl filter tips onto positions B2 and C2. Remove the cover on the tip boxes and close the Workstation Hood.
7.3.13 Right-click on Tip boxes and select **'all tips to available'**.
7.3.14 Then click on **'Control'** and select **'Start'.**
7.3.15 Review the Checklist on the 'pop-up' window (**error reports are indicated in red**) quickly ensuring that:
- Columns you wish to extract from are correctly selected and the unused portion of capture plate is sealed with self-adhesive plastic sealing film or PCR foil.
- Elution plate is in position but not opened yet.
- Sufficient pipette tips are loaded and lids removed.
- Reagent tubs are loaded in their right positions with the correct volumes and tub in place.

7.3.16 Then select all the boxes and click **'OK'** to start the run.
7.3.17 User intervention pause steps are added after each vacuum step. Take the required action and then click **'OK'** to continue the run.
7.3.18 After the initial vacuum step, ensure that all the wells of the Capture plate are completely drained then click **'OK'** to continue. If there is complete blockage of any of the wells, use a 1ml pipette to remove un-drained lysate before continuing to the next step.
7.3.19 At the final user intervention pause step (the drying step after DXF) dispense 8ml of Elution Buffer into a 70ml Reagent tub, cover and load onto Well A2 in position R1 and remove the lid on the Elution Plate.
7.3.20 Close the Hood and click **'OK'** to continue.

7.3.21 At the end of the run, remove the Elution plate and transfer the eluted DNA into a pre-labeled 96-well V-bottom sample storage plates, maintaining the same plate-plan orientation. **Store at -20ºC**.

7.4 POST-RUN CLEANUP

- Dispose of plastic ware and liquid waste in accordance with laboratory guidelines for the sample type and reagent hazard.
- Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste. Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids. For any items contaminated with these buffers, clean with general laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

7.4.1 **Transfer carriages, waste sink and tip chute.**
7.4.2 Thoroughly rinse under cold tap water and allow to dry. Do not apply hot water to these components.
7.4.3 **Separator Plate and Non-disposable Reagent Tubs.**
7.4.4 The Separator Plate and non disposable reagent tubs must be washed to ensure they are RNA/DNA and RNase/DNase-free.
7.4.5 Ensure they are dry before re-using. Do not autoclave or heat sterilize separator plate and non disposable reagent tubs (do not exceed 100°C)
7.4.6 When washing the separator plate, scrub lightly with a brush, this will help dislodge air bubbles that can become trapped in the holes and prevent the plate from been cleaned thoroughly. Agitating the plate up and down will also help ensure the holes are properly washed.
7.4.7 Then soak in 1 % sodium hypochlorite (final concentration of bleach) for >30 minutes and rinse thoroughly with large amounts of Alpha-Q or Molecular Biology grade RNase-free water.

7 Attachments

Attachment number	**Title** (as referenced on the attachment)
N/A	

8 References

Standard Operating Procedure (SOP) for detection of *Plasmodium* infection using Nested PCR Assay.

Date: June, 2010
Version: 1.02

Scope

This SOP describes the procedure for routine diagnostic PCR to detect infection by *Plasmodium* species at the Malaria Diagnostics Unit, MRC (UK) The Gambia.

Background

PCR Diagnosis of Malaria is based on amplifying malaria parasite-specific DNA sequences usually in a nested PCR format involving two rounds of amplification reaction. The target DNA sequence is the 18S rRNA gene which is multi-copy and contains genus and specie-specific variations that are exploited to design discriminatory assays (Snounou *et al.* 1993, Mol Biochem Parasitol 61: 315-20; Rougemont *et al.,* 2004, J. Clin. Microbiol 42: 5636–5643).

Safety considerations

Laboratory coats and gloves should be worn at all times; gloves should be changed at regular intervals.
Ethidium bromide is a mutagen, handle and dispose of with extreme care.
Face and eyes should be protected with a screen or visor when using UV light.

Anti-contamination considerations

DNA extraction, PCR set-up and post-PCR procedures should be carried out in separate, designated areas of the lab. Use of aerosol resistant tips, dedicated pipettes and the frequent changing of gloves also help to prevent cross contamination of samples.

Preparation and storage of reagents

PCR stock reagents (*eg* primers at 100mM) and enzymes should be stored at -20ºC.
Working dilutions of PCR reagents (10X buffer, dNTPs, $MgCl_2$, primers at 10uM) can be stored in the fridge if in frequent use but at -20ºC for longer term storage (ie over one week).
Similarly, working aliquots of sample and control DNA and Nest 1 products can be kept in the fridge if in daily use but must be kept at -20ºC for longer term storage.

Summary of Procedure

- Starting with already extracted DNA* carry out diagnostic PCR as below:
- Nest-1 PCR with primers **rPLU5 + rPLU6**; include positive controls for each species (*falciparum, ovale, malariae & vivax*) and negative control of PCR mix without DNA.
- Run Nest-2 PCR reaction to determine presence of *Plasmodium* infection using the genus-specific primers, **Plasmo-1 + Plasmo-2.**

*See DNA extraction SOP.

- Determine the specific *Plasmodium* species by running four (4) parallel Nest-2 PCR assays (for only positive samples!). Use the primers indicated below to set up separate reaction master mixes to amplify species-specific sequences:
    - *P. falciparum* use **rFAL 1 + rFAL 2**.
    - *P. malariae* use **rMAL 1 + rMAL 2**.
    - *P. ovale* use **rOVA 1 + rOVA 2**.
    - *P. vivax* use **rVIV 1 + rVIV 2**.
- Load 5-10µl of Nest-2 product on a 2% agarose gel containing Ethidium bromide and carry out electrophoresis for 30-45mins at 100v. Photograph the completed gel under UV light using the gel documentation system.
- Score results as presence or absence of bands by comparing with control bands. Two individuals should independently score each gel. Discordant scores should be reviewed by a supervisor and repeated if unresolved.

PCR Protocol

1. Include positive and negative controls into final sample number ensuring that all the species controls are included at the Nest1 stage. A 96-well plate will accommodate 88 samples with one column left for controls.
2. Calculate volume of PCR Master Mix enough for samples +8 to account for pippetting loss and dead volume that will be left in the reaction trough– *eg*. if total number of samples is 80, make enough Master Mix for 88.
3. Make plate plan with detailed notes in the lab book – date, experiment, list of samples to be amplified, target gene for amplification, whether Nest-1 or Nest-2, PCR Master Mix recipe *etc*.
4. Thaw, vortex briefly and spin down all PCR reagents, except Taq polymerase enzyme.
5. Add PCR reagents together in a clean trough in the order listed in the master mix preparation table below. This should be done in a clean, DNA-free environment – preferably a clean, sterile hood with laminar air flow or a designated area. Use dedicated pre-PCR pipettes.
6. Keep Taq polymerase enzyme in an ice box or in the freezer until all other reagents have been added.
7. Mix by pipetting up and down several times with a wide bore pipette tip (P1000) while tilting the trough slightly with the pipette tip fully immersed to avoid foaming.
8. Label clean, sterile PCR plates with sample/N1 or N2/gene/date/operator *etc* as necessary (you can never include too much information!)
9. Dispense Master Mix into the labelled clean, sterile PCR plates according to plate plan (13µl for Nest-1 reactions, 19-29µl for Nest-2 reactions as appropriate).
10. . Spin down DNA or Nest 1 product briefly before opening (quick spin at max speed).
11. . Add DNA (2µl) or Nest-1 product (1µl) to relevant sample according to the plate plan. Seal plate carefully, do a quick spin down and place on PCR machine and start the appropriate cycling programme (see PCR cycling condition below).
12. . After PCR has finished, spin plate briefly. Store in fridge or at -20°C for further use or proceed to gel electrophoresis for Nest-2 reaction product .

Preparation of PCR Master Mix

Outer PCR (Nest I) 15µL

	Stock conc.	Final conc.	1X (µL)	105X (µL)		
PCR H_2O			10.12	1062.6		
Buffer (Thermopol)	10X	1X	1.5	157.5		
rPLU5	10 µM	0.4 µM	0.6	63		
rPLU6	10 µM	0.4 µM	0.6	63		
dNTPs	50 mM	0.2 mM ea	0.06	6.3		
Taq	5 U/µL	0.6 U	0.12	12.6		
DNA			2.0			

Inner PCR (Nest-2) 15µL

	Stock conc.	Final conc.	1X (µL)	105X (µL)		
PCR H_2O Buffer (Thermopol)	10X	1X	11.42 1.5	1199.1 157.5		
Primer 1	10 µM	0.3 µM	0.45	47.25		
Primer 2	10 µM	0.3 µM	0.45	47.25		
dNTPs	50 mM	0.2 mM ea	0.06	6.3		
Taq	5 U/µL	0.6 U	0.12	12.6		
PCR product			1.0			

*Buffer from New England Biolabs; contain $MgCl_2$ at 1.5mM conc

Changes in total volume requirements: as a guide, the volume of *Taq* and primers remains the same. The volume of dNTPs (2.5mM each) and 10x reaction buffer is always 10% of the total volume. Adjust water accordingly. This assumes total volume to a maximum of 50µl per sample.

Sequences of 18s rDNA primers for *Plasmodium* diagnostic PCR

Gene	Primers	Sequence	Product size
18SrRNA	Nest-1 forward rPLU5	5'-CCTGTTGTTGCCTTAAACTTC-3'	1,200 bp
	Nest-1 reverse rPLU6	5'-TTAAAATTGTTGCAGTTAAAACG-3'	
	Nest-2 forward Plasmo 1	5'-GTTAAGGGAGTGAAGACGATCAGA-3'	157-165 bp
	Nest-2 reverse Plasmo 2	5'-AACCCAAAGACTTTGATTTCTCATAA-3'	
	Nest-2 forward rFAL 1	5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3'	205 bp
	Nest-2 reverse rFAL 2	5'-ACACAATGAACTCAATCATGACTACCCGTC-3'	
	Nest-2 forward rMAL 1	5'-ATAACATAGTTGTACGTTAAGAATAACCGC-3'	144 bp
	Nest-2 reverse rMAL 2	5'-AAAATTCCCATGCATAAAAAATTATACAAA-3'	
	Nest-2 forward rOVA 1	5'-ATCTCTTTTGCTATTTTTTAGTATTGGAGA-3'	800 bp
	Nest-2 reverse rOVA 2	5'-GGAAAAGGACACATTAATTGTATCCTAGTG-3'	
	Nest-2 forward rVIV 1	5'-CGCTTCTAGCTTAATCCACATAACTGATAC-3'	120 bp
	Nest-2 reverse rVIV 2	5'-ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3'	

PCR Cycling Conditions:

Nest1:

rPLU5 + rPLU6

- $94°C^{3min}$ / ($94°C^{30secs}$ – $58°C^{30secs}$ – $72°C^{60secs}$ **x24**) / $72°C^{5min}$/End.

Nest2:

Plasmo1 + Plasmo2

- $94°C^{3min}$ / ($94°C^{30secs}$ – $58°C^{30secs}$ – $72°C^{45secs}$ **x29**) / $72°C^{5min}$/End.

Nest2:

rFAL1 + rFAL2; rMAL1 + rMAL2; rOVA1 + rOVA2; rVIV1 + rVIV2

- $94°C^{3min}$ / ($94°C^{30secs}$ – $60°C^{30secs}$ – $72°C^{45secs}$ **x29**) / $72°C^{5min}$/End.

Agarose Gel Electrophoresis

1. Prepare 10X TBE stock buffer as below:

For 1 litre, weigh out:

Tris base	108g
Boric acid	55g
EDTA (di-sodium salt)	9.3g

Add 900mL deionized water, mix on a magnetic stirrer until dissolved. Make up to 1litre in a measuring cylinder.

2. TBE buffer is used at 0.5X concentration for making agarose gels and for use as running buffer. Dilute stock TBE 1:20 (1 part TBE stock + 19 parts de-ionized water) to obtain working concentration of 0.5X

3. Add 0.5X TBE and agarose to a conical flask, mix for a few minutes to hydrate agarose, and melt agarose in microwave.

To make 150ml of 2% agarose gel

0.5X TBE	150ml
Agarose	3g

(Smaller DNA fragments will require higher concentration gels eg 2.5%)

4. Allow to cool until warm to touch while stirring to avoid gelling, add Ethidium bromide (10mg/ml stock solution) to a final concentration of 0.5µg/ml and mix well. Try not to introduce bubbles.
Example; for a 100ml gel, add 5µl Ethidium bromide stock

5. While the agarose is cooling, place gel casting tray on a level surface and seal ends with paper tape or silicone casting gates

6. Insert comb(s) and carefully pour agarose solution into tray.

7. Leave to set for 30mins or longer and then remove ends and combs from gel tray, place in tank and cover gel with 0.5X TBE buffer to a depth of 2-5mm.

9. Mix 2µl of 6X loading buffer with 5-10µl Nest 2 product and load into the well.

10. Load 6µl molecular weight marker (100bp ladder) on every first well followed by the controls and then samples. Follow a particular order for loading samples for each project and note this in the lab book.

11. Connect the electrophoresis plate and run at approx 3-5volts/cm (100v for a 20cm gel) for 30 mins-1hour (depending on degree of separation required)

12. Visualise bands by placing gel on UV transilluminator. Capture gel photo using the geldoc system, save and print a copy of gel picture for scoring.

Nested PoTRA protocol for distinguishing between P.o. curtisi and wallikeri

NEST 1

Reagent	Final Concentration	Volume
Nuclease-free H_2O		8.0µl
10X NH_4 Buffer	1X	2.0
50mM $MgCl_2$	2mM	0.8
10mM dNTPs	1.0mM	2.0
10µM PoTRA fwd 3	0.5µM	1.0
10µMPoTRA rev 3	0.5µM	1.0
5U BioTaq	1U	0.2
DNA		5.0
Total volume		20.0

NEST 2

Reagent	Final Concentration	Volume
Nuclease-free H_2O		12.0
10X NH_4 Buffer		2.0
50mM $MgCl_2$		0.8
10mM dNTPs		2.0
10µM PoTRA fwd 5		1.0
10µM PoTRA rev 5		1.0
5U BioTaq		0.2
1° Amplicon		1.0
Total volume		20.0

Primer sequence

Cycling conditions

PCR Primer sequences and reaction conditions used in PoTRA and PoRBP2 amplification reactions

Gene	Primer name	Primer sequence	PCR product size(bp)	PCR cycling conditions
PoTRA	PoTRA fwd3 PoTRA rev3	5'-GCACAAAAATGGTGCTAACC-3' 5'-ATCCATTTACCTTCCATTGC-3'	787	95°C for 2min ; (95°C for 30s ; 58°C for 45s ; 72°C for 1min) x 35cycles ; 72°C for 5min
	PoTRA fwd5 PoTRA rev5	5'-ACGGCAAACCCGATAAACAC-3' 5'-GTGTTTGTAGTATTTACAGG-3'	299bp	95°C for 2min ; (95°C for 30s ; 52°C for 45s ; 68°C for 1min) x 29cycles ; 68°C for 5min

PCR product size: Poc 299bp; Pow 245bp.

Note: There is another Poc with 6 extra repeats about 319bp.

Run on 1.5% agarose gel stained with ethidium bromide and run at 120V

APPENDIX 3

Participant Information Sheet and Consent form

Title: Intermittent preventive treatment with sulphadoxine-pyrimethamine versus intermittent screening and treatment of malaria in pregnancy

Investigators: Local Investigators

The following will be translated into appropriate local languages.

Malaria in pregnancy is life threatening to both the mother and the baby she is carrying. It can result in the destruction of the mother's blood and in babies born weighing less than normal, making them less healthy in their first years of life. Over the years, effective measures have been taken to control the harmful effects of malaria in pregnancy and this has resulted in less pregnant women suffering from malaria than previously in our country, as in many parts of Africa. Scientists now think it is time to find out whether one of these measures called intermittent preventive treatment (IPTp), which requires that pregnant women are given a malaria drug called SP on several occasions during pregnancy, is still useful particularly in areas like ours where pregnant women are at risk for only a short period of the year.

We in Burkina Faso, Ghana, Mali, and the Gambia wish to test this idea in two groups of pregnant women. One group will have a blood test for malaria and treatment of anyone who is positive at scheduled antenatal clinic visits; the second group of pregnant women will be given the drugs two or more times as is currently done. When women in the two groups deliver we will compare the strength of their blood and the weight of their babies and take a sample of the placenta to see if this has been infected with malaria. This will help us to know which strategy is more effective in protecting pregnant women from having low birth weight babies and anaemia due to malaria infection. We would like you to help us do this by participating in the study. If you agree to participate in this study, you will be one of 5000 women in Ghana, Burkina Faso, The Gambia and Mali also participating in the study. The findings will help the Ministry of Health to better control malaria in pregnancy.

If you agree to participate in this study, this is what this will involve.

1. We will ask you some questions about yourself including your general health, your education level and living conditions.
2. We will take a little blood by a prick of your fingertip to test for malaria and thinning of blood to help us know your health status at the beginning of the study. The blood samples will not be used for any other purpose without your permission.

3. You will be allocated to one of the two options by lottery. You will receive a long-lasting insecticide treated bed net and will be expected to use it throughout your pregnancy. We will visit your home at random to find out whether you are using it.
4. If we find malaria parasites in your blood you will be given treatment. You will be required to return to the clinic on dates we give you.
5. If you are included in the group that requires us to test you for malaria and treat if this is found on the blood test, we will do this on the dates we give you. If you are allocated to the group to receive treatment as is currently practiced you will be given the next dose of SP.
6. We will ask you to deliver at hospital so that we can examine and weigh your baby, check the thinning of your blood and obtain a sample of your placenta. However, if you are unable to deliver at hospital please notify us through a member of your community health committee so that we can visit you at home and do the necessary tests on you and your baby as soon as possible after it is born.
7. When your baby is 40 days old we will visit you to find out how you and the baby are doing and take blood by finger prick to test for malaria parasite and thinning of blood. This visit marks the completion of your participation in this study.

From today until the time you deliver you will not be required to pay for any treatment you receive as part of this study. This includes other visits you may make for other illnesses on days other than those we gave you. Every medicine including those for treating malaria and strengthening your blood will be free. There will be someone at the clinic to attend to you anytime you come in between scheduled visits.

Any information we have about you will not be disclosed to anyone by us. Participation in this study is voluntary. If you do not wish to participate in the study, it will not affect the regular attention you have been receiving from this hospital/health centre now or in the future. You may withdraw from the study at any time without this affecting the care that you receive at the antenatal clinic.

We are happy to answer any questions you may have that will help you decide whether or not you want to take part in the study, or to explain further what taking part in this study will mean for you and your baby.

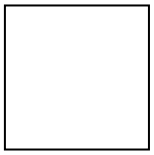
CONSENT:

I have been adequately informed of (I have read and understood) the purpose, procedures, potential risks and benefits of this study. I have had the opportunity to ask questions about the study. Any questions that I have asked have been answered to my satisfaction. I know that I can refuse to participate in this study without any loss of benefit to which I would have otherwise been entitled. I understand that if I agree to participate, I can withdraw my consent at any time without losing any benefits or services to which I am entitled. I understand that any information collected will be treated confidentially. I understand that agreeing to participate and signing this form does not necessarily mean I will be able to participate in the study because I will be assessed for eligibility before enrolment.

I freely agree to participate in the study. After signing below, I will receive a copy each of the information sheet and the consent form.

Name of participant:
…….

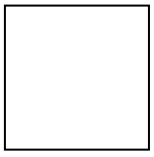
Signature or Right Thumb Print: ……



Date: -----/-----/-----

Name of witness:
……..

Signature or Right Thumb Print: ……..



Date: -----/-----/-----

I have adequately informed the participant of the purpose, procedures, potential risks and benefits of this study. I have answered all questions to the best of my ability.

Name of study personnel: ……..

Signature: ……

Date: -------/------/------

ASSENT FORM FOR PREGNANT WOMEN UNDER LEGAL AGE

(To be completed by the woman and their parent/guardian)

Under age woman (or if unable, parent on their behalf) /young person to circle all they agree with:

Have you read (or had read to you) about this project?	Yes/No
Has somebody else explained this project to you?	Yes/No
Do you understand what this project is about?	Yes/No
Have you asked all the questions you want?	Yes/No
Have you had your questions answered in a way you understand?	Yes/No
Do you understand it's OK to stop taking part at any time?	Yes/No
Are you happy to take part?	Yes/No
If <u>any</u> answers are 'no' or you **don't** want to take part, **don't** sign your name!	

If you <u>do</u> want to take part, please write your name and today's date

Your name _____ Age_____

Date _____

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name _____

Sign _____

Date _____

The doctor who explained this project to you needs to sign too:

Print Name _____

Sign _____

Date _____

Thank you for your help.

INFORMED CONSENT FORM FOR PARTICIPANTS UNABLE TO GIVE CONSENT THEMSELVES

	Please initial box
1. I confirm that I have read and understand the subject information sheet dated (please insert date) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully.	☐
2. I understand that my child/spouse/partner's participation is voluntary and she is free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected.	☐
3. I understand that sections of any of their medical notes and data collected during the study, may be looked at by responsible individuals from (please insert centre name), from regulatory authorities and sponsors to this study, where it is relevant to my taking part in this research. I give permission for these individuals to access my records.	☐
5. I agree for my child/spouse/partner to take part in this study.	☐

_____	_____	_____
Name of Participant	Signature (if able)	Date
_____	_____	_____
Name of parent/guardian/legal representative	Signature	Date
_____	_____	_____
Name of Person taking consent (if different from Principal Investigator)	Signature	Date
_____	_____	_____
Investigator	Signature	Date

1 copy for subject and to be kept with participant's CRFs