ASSESSMENT OF MALARIA RAPID DIAGNOSTIC TEST AND UTILISATION OF LABORATORY SERVICES FOR MALARIA DIAGNOSIS AT MAKARFI GENERAL HOSPITAL, KADUNA STATE

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AUGUST, 2012

DECLARATION

I declare that the work in the dissertation entitled "Assessment of Malaria Rapid Diagnostic Test and Utilisation of Laboratory Services for Malaria Diagnosis at Makarfi General Hospital, Kaduna State" has been performed by me in the Department of Community Medicine under the supervision of Prof. K. Sabitu and Dr A. A. Aliyu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

Dr Ajumobi Olufemi Olamide	Signature	Date

CERTIFICATION

This thesis entitled, "**ASSESSMENT OF MALARIA RAPID DIAGNOSTIC TEST AND UTILISATION OF LABORATORY SERVICES FOR MALARIA DIAGNOSIS AT MAKARFI GENERAL HOSPITAL, KADUNA STATE**" by **AJUMOBI, Olufemi. Olamide** meets the regulations governing the award of the degree of Master in Public Health of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

In Nigeria, malaria accounts for 30% mortality in children <5years (U5). World Health Organisation guidelines recommend parasite-based diagnosis prior to commencement of antimalarial treatment. However, empirical treatment remains a common phenomenon at homes and in clinical settings because of presumed high malaria prevalence. This is compounded by inadequate information on accuracy of malaria rapid diagnostic test (RDT). Data on factors affecting the utilisation of malaria laboratory services (MLS) is not readily available. We conducted a study to assess the diagnostic accuracy of Standard Diagnostic (SD) Bioline malaria rapid diagnostic test, and to determine the factors affecting the utilisation of MLS in febrile children in Kaduna State, Northern Nigeria.

We conducted a cross-sectional study of 296 caregivers of febrile U5 at Makarfi General Hospital (MGH), Kaduna state from December 2010 to August 2011. We used structured questionnaires to collect data on socio-demographics of caregivers, clinical information on febrile U5, and utilisation of MLS. Blood samples were collected from 296 U5 and examined for malaria parasites with SD Bioline rapid diagnostic test (RDT) and routine microscopy. The diagnostic accuracy of the RDT was determined. Focus group discussions (n=4) were conducted at Makarfi communities to determine the factors affecting utilisation of MLS among caregivers of children U5. Eight key informants were interviewed to determine the capacity for malaria diagnosis at MGH.

The sensitivity, specificity, negative and positive predictive values of SD Bioline RDT were 100%, 98.5%, 100% and 88.6% respectively. The prevalence of malaria in the febrile children was 10.5% and 11.8% by RDT and microscopy respectively. *Plasmodium falciparum* infection rate was 100%. Overall, 3.7% of caregivers were offered any malaria laboratory test by health staff and 5.5% have ever heard about MLS. Non-request of MLS by health staff, lack of awareness, presumptive treatment, and long distance from health centre, caregiver's perceived severity of illness, high cost, and non-availability of MLS were responsible for poor utilisation of malaria laboratory services. Lack of trained staff, basic reagents for malaria microscopy and quality assurance mechanisms were responsible for poor capacity for malaria diagnosis at MGH.

SD Bioline RDT has a high sensitivity and specificity despite low prevalence of malaria. This strongly precludes the current practice of presumptive treatment of fever in U5. SD Bioline RDT should be rapidly deployed to all health facilities where there are no facilities for microscopy. Kaduna state and Makarfi local government should sensitise health care providers on confirmatory malaria diagnosis in children. They should sensitise caregivers in rural communities about utilisation of MLS. Equally, they should provide access to MLS at an affordable cost in rural communities and urgently strengthen capacity for MLS.

Key words: malaria, sensitivity, laboratory services, rapid diagnostic test, Nigeria

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ABBREVIATIONS

AA	Artesunate-Amodiaquine
ACT	Artemisinin Combination-therapy
AFENET	African Field Epidemiology Network
AL	Artemether-Lumefanthrine
CDC	United States Center for Disease Control and Prevention
CFR	Case Fatality Rate
CRF	Case Report Form
FGD	Focus Group Discussion
FMoH	Federal Ministry of Health
G6PD	Glucose 6-Phosphate Dehydrogenase
GFATM	Global Fund to fight AIDS, Tuberculosis and Malaria
HRP	Histidine-Rich Protein
IDSR	Integrated Disease Surveillance and Response
IRIN	Integrated Regional Information Networks
LGA	Local Government Area
MDG	Millennium Development Goals
MGH	Makarfi General Hospital
MIM	Multilateral Initiative on Malaria
NFELTP	Nigeria Field Epidemiology and Laboratory Training Programme
NMCP	National Malaria Control Programme
N.B.	nota bene (latin for “note well”)
NPV	Negative Predictive Value

PCR	Polymerase Chain Reaction machine
P.f	*Plasmodium falciparum*
pLDH	*Plasmodium* Lactate Dehydrogenase
P.m	*Plasmodium malariae*
P.o	*Plasmodium ovale*
PPV	Positive Predictive Value
P.v	*Plasmodium vivax*
RDT	Rapid Diagnostic Test
STARD	Standard for Reporting Studies of Diagnostic Accuracy
U5	Children less than 5 years of age
UNDESA	United Nations Department of Economic and Social Affairs
UNICEF	United Nations Children's Fund
WHO	World Health Organization
WHO/TDR	World Health Organization/Tropical Disease Research

CHAPTER ONE: INTRODUCTION

1.1 Background

Malaria is a disease of the tropics and sub-tropics and is transmitted by a vector, female *Anopheles* mosquito. It is caused by *Plasmodium (P.) falciparum, P. malariae, P. ovale and P. vivax.* In Nigeria, the prevalent species is *Plasmodium falciparum* accounting for >90% of all diagnosed cases (95-98%), *P. ovale* (<2%), *P malariae (2-5%)* while *P. vivax* is not endemic in Nigeria. The dominant vector species are *Anopheles (An) gambiae s.l.* and the *An. funestus*.[1-3] Malaria is a public health problem in Nigeria and accounts for 110 million clinically diagnosed cases per year. The disease is responsible for about 60% outpatient care-visits and 30% hospitalizations. At least 300,000 children die of malaria per year in Nigeria and up to 30% childhood deaths, 25% of deaths in children under one year and 11% maternal deaths are due to malaria.[4, 5] It is estimated that 50% of the population will have at least one episode of malaria annually, with 2-4 episodes in under-5 year old children.[3] An estimated N132 billion is lost due to malaria annually in form of treatment costs, prevention, loss of man hours.[4, 5] In 2008; 9,591 deaths were reported out of 3,481,220 reported cases of malaria in Nigeria, giving a Case Fatality Rate of about 0.3% compared to 0.2% in 2001.[6] On a global scale, in 2008 there was an estimated 243 million cases of malaria worldwide with the majority of cases (85%) in the African Region, followed by the South-East Asia (10%) and Eastern Mediterranean Regions-4%.[7]

Over-prescription of antimalarial medicines is a very common phenomenon because the diagnosis of malaria is often presumptive, despite recommendation of routine laboratory diagnosis of malaria by the World Health Organisation (WHO).[8] The use of laboratory methods

has become necessary because health workers cannot identify malaria cases reliably using clinical signs and symptoms alone.[9, 10] Conventional light microscopy of a blood smear is the reference gold standard for the detection of malaria parasites and the established method for the laboratory confirmation of malaria. It is sensitive and can detect densities as low as 5–10 parasites/μL of blood when used by skilled and careful technicians,[11] but realistically at 100 parasites/μL under field conditions.[12] Microscopy provides information on parasite species (*P. falciparum*, *P. vivax*, *P. ovale*, and/or *P. malariae*) and their circulating stages (e.g. trophozoites, schizonts, gametocytes). It aids quantification of the parasite densities and assessment of parasitological response to chemotherapy in severe malaria cases. It can provide a permanent record (the smears) of the diagnostic findings and be subjected to quality control. However, malaria diagnosis based on microscopy is labour-intensive and time-consuming, requiring at least 60 minutes from specimen collection and availability of result. Long delays occur in providing microscopy results to the clinician and as such, decisions on treatment are often taken without the benefit of the results.[13] Microscopy depends absolutely on good techniques, reagents, microscopes and, most importantly, well trained and well supervised technicians. These conditions are hardly met at the more peripheral levels of the health care system making microscopic diagnosis an unreliable tool.

Rapid diagnostic tests (RDT) are recommended by WHO to enhance diagnosis and management of cases, prevention of complications of delayed treatment, prolonging survival and monitoring of treatment especially in children. Moreover, studies have shown that it is acceptable both to the practising physicians and the patients.[8] It is timely for case management of malaria and avoids the drawbacks of defective microscope and erratic power supply.[8] It aids selective treatment of

only patients with positive dipstick results and thus slows down the development of drug resistance by preventing drug pressure.[14] Health workers with minimal skills can be trained in RDT techniques within periods varying from three hours to one day.[13, 15] RDT is robust, non-cold chain dependent and the degree of variability of test reliability and performance among individual users is relatively small.[13] The specificity and sensitivity of 97% and ≥95% respectively have been shown, for self-diagnosis among travellers from endemic areas returning to United Kingdom and in remote areas of Philippines.[16, 17]

Malaria RDT detects serum antigens of *Plasmodium* species using fixed antibodies on strips of paper. There are three types of newly developed RDTs on the WHO pre-qualified list. According to WHO, *Plasmodium* Lactate Dehydrogenase (pLDH) based-tests can detect all the *Plasmodium* species that infect humans.[13] They can distinguish *P. falciparum* from the non-*falciparum* species, but cannot distinguish between *P. vivax*, *P. ovale* and *P. malariae* (i.e. specific for *P. falciparum).* Some tests detect Aldolase antigens of *Plasmodium malariae, ovale* and *vivax or* specifically that *of P. falciparum* while others detect *Plasmodium falciparum* Histidine-Rich Protein II (HRP 2) antigen.[18-20] Other antigen(s) that are present in all four species are also targeted in kits that combine detection of the HRP-II antigen of *P. falciparum* together with that of an, as yet unspecified, "pan-malarial" antigen of the other species.[13] The choice of RDT depends on the malaria endemicity and species prevalence.[21]

In 2009, WHO recommended that persons of all ages with suspected malaria cases should undergo rapid diagnostic tests, but only 18 of 35 countries reported adhering to this.[7] In 18 malaria high-burden African Region countries for which data were available, 22% of the

reported suspected malaria cases were confirmed with RDT in 2008.[7] The validity of some RDTs has been investigated over the years and has been shown to be of high specificity and sensitivity in comparison to light microscopy.[17, 22] The WHO recommends a sensitivity of ≥95% at ≥100 parasites/µl for *P. falciparum*.[13, 22] Despite this recommendation and the reported validity of RDT, recent field and clinical-based studies indicated a low specificity and sensitivity compared with routine microscopy. The reasons alluded to were low parasitaemia, defective and inappropriate handling of RDT kits.[7, 22-28] The National Malaria programme has commenced the "roll out" of rapid diagnostic tests in some health facilities across the country, with the aim of scaling up its use in the field, where there is lack of skilled laboratory scientists and less than optimal conditions for routine microscopy. The recent trend of increasing mortality due to malaria in the general population and in high risk groups of pregnant women and children under the age of 5 years necessitates a more rapid scale up of malaria RDT.[6]

There is a high incidence of malaria in Nigeria with variability in endemicity. The southern part of Nigeria and the lower part of the northern region are associated with endemic and perennial episodes (7-12months). Transmission is endemic and seasonal (4-6months) in the upper north and epidemic or strongly seasonal in the extreme north-eastern part of the country.[3] The proportion of malaria confirmed by laboratory diagnosis in Nigeria is unknown. Diagnosis of malaria is often clinical-based and unreliable.[8-10] This could lead to over diagnosis, inappropriate treatment and potential development of drug resistance. The use of routine laboratory microscopy to aid clinical diagnosis is minimal. However, the use of malaria rapid diagnostic tests is a new approach and its implementation is restricted to a few facilities in the country. Artemisinin-based combination therapy, the currently recommended treatment for malaria

though efficacious, is costly. There is an urgent need to prioritise its use for cases that are purely due to *P. falciparum* because of possibility of potential development of drug resistance. This study aims at ascertaining the sensitivity, specificity, and predictive value of standard diagnostic (SD) Bioline malaria rapid diagnostic test in relation to routine light microscopy.

1.2 Statement of the problem

Diagnosis based on microscopy requires laboratory expertise which is not always available at the periphery. The time span between sample taking and availability of results is often too long. This and occasional unreliability of the results allow for presumptive diagnosis of malaria cases by physicians. Presumptive diagnosis often leads to over-diagnosis and institution of empirical treatment of febrile cases by physicians. The treatment of non-malaria febrile episodes can result in, and have been linked to drug resistance. The drug therapeutic efficacy studies carried out in six epidemiological regions of the country in 2002, revealed growing resistance to commonly used antimalarials, which were monotherapies namely Chloroquine (CQ) and Sulfadozine-Pyrimethamine (SP).[4] These studies showed that CQ and SP, the cheap and affordable antimalarials; were no longer efficacious for first line treatment of uncomplicated malaria in Nigeria[4]. The indiscriminate treatment of presumptive malaria without parasitological diagnosis can result in potential drug resistance to the available anti-malarial drugs which are new and are expensive and progression to severe malaria despite treatment. In line with WHO guidelines regarding the review of the malaria treatment policy, which states that when total treatment failure is ≥10%, the national anti-malarial treatment policy guideline was reviewed in 2005. [30] In order to reduce the increasing mortality associated with severe malaria, there is a need for early and focused diagnosis. This has led to the introduction of malaria rapid diagnostic test.

However, there are few studies on the sensitivity, specificity, and predictive value of the RDTs when used in clinical settings in endemic areas in northern Nigeria. Furthermore, studies that have addressed variation in pattern of malaria transmission in Nigeria are at best few. [2, 27, 30]

In a conversation with I. M. Watila, MD (July 2010); it was noted that in recent years, "the influence of the epidemiological situation (i.e. the proportion of *P. falciparum* and other *Plasmodium* species in febrile children less than 5years) on the rapid test result is not known". Existing indigenous studies are limited.[27, 31] Kaduna state is situated in the lower part of the north-western zone, associated with endemic and perennial episodes of malaria.[3] There is a growing concern about the accuracy of malaria RDT results. Its usefulness in providing informed decision on malaria case management has been questioned by health care workers. To our knowledge, no study has been done in Kaduna state to ascertain the validity of RDT in parasitological diagnosis of malaria in a clinical setting.

Acceptance of the use of laboratory services should precede malaria diagnosis, laboratory testing before diagnosis and diagnosis before treatment. Lack of utilisation of laboratory services for confirmation of malaria diagnosis among cases of febrile illnesses can result in missing cases of malaria and overdiagnosis, potential development of drug resistance, and mismanagement of febrile cases. Data on factors affecting the utilisation of laboratory services for malaria diagnosis in Nigeria are not readily available.

1.3 Rationale

Efficacy studies conducted by the Federal Ministry of Health on resistance and treatment failure associated with CQ and SP in 2004, showed two suitable Artemisinin based-combination therapies (ACTs) namely Artesunate–Amodiaquine (AA) and the Artemether-Lumefanthrine (AL). AA and AL have been adopted as the first line drugs for the treatment of uncomplicated malaria in Nigeria.[5] However, these drugs are expensive and not affordable by an average Nigerian. Affordable medicine facility for malaria (AMFm) - an initiative that seeks to ensure distribution of low cost ACTs is relatively new in Nigeria. There is need for focused diagnosis and treatment of malaria cases to prevent resistance to the current highly efficacious drugs, given their anticipated wide distribution through AMFm. Development of resistance to the available antimalarial drugs can portend danger to the control of malaria which is an endemic disease in Nigeria. The use of laboratory services by the clinician and patient/guardian is vital. Rapid availability of valid laboratory results will assist the clinician to decide on treatment. Therefore parasite diagnosis should precede the use of current antimalarials. Accurate malaria diagnosis can be achieved with highly sensitive and specific RDT. It is important to establish the diagnostic accuracy of malaria rapid tests for accurate diagnosis and commencement of appropriate treatment. This study will provide some evidence along this line.

The knowledge of sensitivity, specificity, and predictive value of the available RDTs is crucial for early diagnosis of malaria in febrile children less than 5 years, using rapid tests of high diagnostic accuracy in a clinical setting in endemic areas. The result of this study will give evidence-based information on sensitivity, specificity and predictive value of SD Bioline RDT in comparison to gold standard. It will provide information on the proportion of different

Plasmodium species seen in febrile children presenting at a hospital in northern Nigeria. In addition, this study will provide information on factors affecting the utilisation of laboratory services for malaria diagnosis in Nigeria. The perceived benefits of the aforementioned can trigger/influence a policy change that would address these factors in vulnerable groups.

1.4 Scope of the study

This study focused on patients presenting with fever or history of fever at the health facility. It addressed the sensitivity, specificity and predictive values of SD Bioline RDT, and the associated parasite-specific factors in febrile children below 5 years (6months - 59months) who presented at Makarfi General Hospital, Kaduna state (MGH). In addition, this study addressed factors affecting utilisation of malaria laboratory services in caregivers of these children, in members of staff of MGH the health facility, Makarfi and Kuruntumawa communities within Makarfi town.

1.5 Objectives

1.5.1 General Objective

To assess the diagnostic accuracy of SD Bioline malaria rapid diagnostic test and utilisation of laboratory services for malaria diagnosis in febrile children at Makarfi General Hospital in Kaduna state.

1.5.2 Specific objectives

1. To determine sensitivity, specificity, and predictive value of RDT for detection of *P. falciparum* using microscopy as reference standard.
2. To determine factors influencing sensitivity, specificity, and predictive value of a RDT for *P. falciparum* (such as parasitaemia and species).
3. To determine the proportion of the different *Plasmodium* species in febrile children under the age of 5 years.
4. To determine factors affecting the utilisation of malaria laboratory services by caregivers of febrile children attending Makarfi General Hospital and members of Makarfi community.
5. To determine the capacity of laboratory staff (experience, skills, and training) at Makarfi General Hospital for malaria diagnosis.
6. To make recommendations for public health action based on the findings from above.

CHAPTER TWO: LITERATURE REVIEW

Malaria is a disease commonly found in the tropics and sub-tropics where climatic conditions favour the survival of *Anopheles* vectors. There are four species that commonly infect humans viz; *P. malariae (P.m), P. ovale (P.o), P. vivax (P.v) and P. falciparum (P.f). P.v* is not reported in Africans because they lack the duffy antigen which is necessary for the infectivity of Anopheles. However, recent studies have given anecdotal evidence to the contrary. There have been some sporadic cases of *P.v* in Africans. *Plasmodium falciparum* is the predominant specie in the world. It is endemic in Africa where it is responsible for >90% of malaria cases; both uncomplicated and severe. In a conversation with N.G. Ntadom, M.D (June, 2010), recent reports alluded to resurgence *of P.o* in Ghana. This is a source of concern because it has implications for drug therapy. Primaquine, is the drug of choice singly or in combination for total clearance of the liver hypnozoites. However, it causes hypersensitivity reaction in patients with G6PD enzyme deficiency, from which about 15% of Nigerians suffer.

Female *Anopheles* mosquito is the major vector of transmission of malaria in humans. It has approximately 460 recognized species, 100 of which transmit human malaria and 30-40 of which transmit *Plasmodium* parasite. *Plasmodium falciparum* causes malaria in humans in endemic areas and is the most dangerous malaria parasite species. Over 40 species of *Anopheles* have been identified in Nigeria.[32] *Anopheles (An.) gambiae* is the best known vector of malaria and is the most efficient transmitter of malaria in Nigeria and *An. arabiensis* as the predominant sibling species of the *An. gambiae* complex.[33-35] The discovery of the mode of transmission of malaria is attributed to the efforts by Ross and Grassi. Ronald Ross (1857-1932) while working in India and Giovanni Battista Grassi (1854-1925) working in Italy discovered the mode of transmission

of malaria[36]. In August 1898, Grassi discovered that the vector responsible for transmitting malaria to man is the female *Anopheles* mosquito.

2.1. People at risk of severe form of malaria

Malaria is not restricted to any age group. Commonly, children below the age of five years (U5) are mostly affected and are prone to high parasitaemia.[27, 31, 37] In a study carried out at the Maiduguri University Teaching Hospital, Borno state Nigeria, of the 692 children screened over a one-year period; Sixty eight point six percent (68.6%) of 169 infected persons were aged 12-59 months with a malaria prevalence of 76.3%.[38] This was corroborated in a study at the Federal Medical Centre, Yola which found a prevalence of 76.4% in this age group.[27] Additionally, this pattern is replicated based on endemicity. In areas of high transmission (such as sub-Saharan Africa), young children are most affected and are at risk of severe malaria. However, in areas of low transmission (such as Latin America and Asia), though cases are fewer, all age groups are affected but less frequently and with less severity.[39] In the same vein, vulnerable groups include those with little or no immunity against the disease. These risk groups include: pregnant women, people living with HIV, travelers/migrants from non-malaria endemic regions, as well as individuals in complex emergency situations and epidemics.[29, 40] These groups of individuals are equally prone to severe forms of malaria e.g. cerebral malaria and malaria with complications: severe anaemia, black-water fever, renal damage, etc. This is especially fatal in children below five years.[29, 41] Failure to initiate early treatment has potential for progression to severe forms of the disease with attending high morbidity and mortality.[42]

2.2. Malaria diagnostic techniques and trends

The traditional and conventional method of diagnosis is light microscopy. It entails preparation of thin and thick blood films and staining with Field's Wright's or Giemsa stains[43]. The thin film enables the determination of parasite density and identification of species and has a better specificity compared to the thick films. The thick film is more sensitive, enables detection of parasitaemia at lower levels, and during recrudescence or relapse of infection.[26, 43] However, preparation of blood film is laborious, demands expertise and is subject to observer interpretation at varying levels of parasitaemia. The latter affects its sensitivity.[44] Though microscopy is less sensitive compared to newer methods, it remains the acceptable gold standard because of the need to stick to a consistent format considering low resource settings. Microscopy has an accuracy of 70-75%.[45, 46] The need for improvement in the diagnostic accuracy i.e. sensitivity and specificity of light microscopy in the detection of parasites led to the use of fluorescent dyes (fluorescent microscopy) and concentrated thick film (quantitative buffy coat technique).[47] This affords a specificity of ≥93% for infections with *P. falciparum* and ability to recognize the small ring forms[48]. Recently, there has been an improvement in time span for light microscopy shortening this to about 5minutes.[49]

"The major advantages of using a PCR-based technique are the ability to detect malaria parasites in patients with low levels of parasitaemia and identify them to the species level. Infection with five parasites or less per µl can be detected with 100% sensitivity and equal specificity".[50, 51] PCR-based methods are particularly useful for studies on strain variation, mutations, and studies of parasite genes involved in drug resistance.[26] In a study that used PCR as a gold standard, the specificity of expert microscopy (EM)>health centre microscopy (HCM)>RDT while sensitivity is in reverse order (with HCM≈EM).[52]

2.3. Presumptive diagnosis and misdiagnosis of malaria and their implication

In a study carried out in Gwagalada, Abuja, Nigeria; only 63.6% of persons who had febrile episodes in the previous month had laboratory confirmation (microscopy).[53] Traditional malaria diagnosis is empirical i.e. presumptive. Its specificity is about 21% when diagnosis is based on fever alone and 42% when used in combination with nail bed pallor and splenomegaly.[54, 55] Thus, about 80% of cases are treated blindly with implication for drug wastage and potential drug resistance. The cost implication of this is enormous. "For every billion dollars in subsidy for antimalarial drugs, around $500m to $960m will be spent on treatment for people who do not have malaria".[56] Rapid Diagnostic Test has been found to be more cost saving than other malaria diagnostic strategies.[57] It is more cost effective to use RDT than to use presumptive treatment and microscopy.[57] In addition, "misdiagnosis of malaria results in excessive reporting of malaria cases, under-reporting of diseases that mimic malaria symptoms, increased true or perceived malaria resistance, and misallocation of resources".[58] "Malaria misdiagnosis will result in more clinic attendances, putting additional pressure on already constrained and under-resourced health systems".[59]

The use of reliable malaria RDTs will help to prevent over-diagnosis, empirical treatment, and misdiagnosis by physicians. It will also enable focused diagnosis and treatment of malaria in children and prevent drug wastage, avoidable adverse drug effects, and development of resistance to the currently available antimalarial drugs.[60] Emergence of drug resistance is one of the greatest worry for the malaria research community**.** Resistance to the previous standard treatment for malaria Chloroquine was first reported in the 1950s in the Thai-Cambodia border and by the 1980s it has spread to sub-Saharan Africa. In 2007, evidence of resistance to

Artemisinin emerged from Southeast Asia, once again at the Thai-Cambodia border, and in 2011 in eastern Myanmar.[61] Rapid Diagnostic Test ensures rational use of ACTs, ultimately preventing drug resistance.[62] Early diagnosis fosters early and effective treatment, and reduces the risk for severity of malaria in vulnerable groups.[42] Treatment with ACT aids depopulation of gametocytes (sexual forms) and therefore reduces transmissibility of malaria parasites.

2.4. Malaria Rapid Diagnostic Tests: sensitivity and specificity

Rapid Diagnostic Test works based on immunochromatographic principle, with the formation of antigen-antibody complexes with the specific malaria antigen released from lysed blood. The specific malaria antigen is identified as a procedural line on the test strip. The nitrocellulose strip is coated with specific antibody for a particular species or all species (pan). Colour-coded control and procedural lines are formed on the introduction of a buffer solution to the antibody wells.[13, 26]

"Histidine-rich protein 2 (HRP-2) is a water-soluble protein produced by trophozoites and young gametocytes of *P. falciparum* while parasite lactate dehydrogenase (pLDH) is produced by asexual and sexual stages (gametocytes) of all malaria parasites".[13] HRP-2 based RDT detects HRP of *P. falciparum* and the pLDH RDT detects all four species in humans. It can thus distinguish *P. falciparum* from non-falciparum species (*P. malariae, ovale or vivax*) but cannot distinguish between these latter species. The choice of the RDT by each country/geographical area depends on level of endemicity, prevalence and type of drug resistance, geographical accessibility, social and economic characteristics, and underlying health infrastructure.[13]

WHO recommends a sensitivity of ≥95% at ≥100 parasites/µl for *P. falciparum*.[13, 23] This depends on RDT product in use as well as its mechanism of action. This could vary whether it is used in clinical or field settings. In a study conducted in Yola, Adamawa state [27] reported a sensitivity and specificity of 69.7% and 100% respectively for Global device that detects pLDH of *P. falciparum*. The negative and positive predictive values were 62.9% and 100% respectively. Using SD Bioline Malaria antigen test kits, which detect all *Plasmodium* species, in a study of 98 hospital patients in Bangladesh [63] a sensitivity of 93.2% and specificity of 94.9% using microscopy as gold standard were found. There was a disparity in the rate of detection of malaria by the rapid test and microscopy which were 58.2% and 60.2% respectively. In a study conducted in three government designated public hospitals in Tanzania at peak malaria season of January to March (low transmission areas) and June – August (high transmission areas) used Paracheck a *P. falciparum* HRP-2 specific RDT revealed a sensitivity of 95.4% and specificity of 95.9%.[8] In a similar study conducted at primary health care centres in rural Madagascar [64], a sensitivity and specificity of 93% and 98.9% respectively for SD Bioline Ag Pf test and 92.9% and 98.9% for SD Bioline Malaria Ag Pf/Pan test were found. A sensitivity of 93.5% was found for Parasight F dipstick for *P. falciparum,* used in screening patients at a primary health care centre in Thailand.[15]

In a study carried out at the hospital for Tropical diseases, London, using pLDH specific RDT for *P. falciparum* and the pan-specific pLDH for *P. falciparum* and *P. vivax*, a sensitivity and specificity of 95.3% and 96% for *P. falciparum* and 96% and 100% for *P. vivax* were reported.[25] In a study conducted in a similar setting (non-endemic) reported a sensitivity of 91.6% for *P. falciparum* (*P. falciparum* specific HRP-2 RDT) and 75.8% for *P. vivax (P. vivax* specific pLDH

RDT) [65]. However, a sensitivity of 42.3% for a *P.f* RDT in a study conducted among eighty-three febrile children at the Paediatrics clinic, University of Nigeria Teaching Hospital, Enugu, Nigeria was found[66]. In field settings, a sensitivity of 47.5% has been found using Parascreen RDT [28].

2.5. Factors influencing sensitivity, specificity, and predictive value of a RDT for *P. falciparum*

Several factors could affect the diagnostic accuracy of RDT. These can be broadly categorised into test device related factors (quality control/assurance, storage, transportation, handling, environmental conditions), preparation and interpretation issues (volume of blood and buffer, age and storage of blood sample, visual acuity of technician), and parasite-specific (parasite prevalence, parasite antigen, density and species).[67, 68] Others include host related factors such as the treatment history and effectiveness of treatment.[67] Interpretation of RDT results should take into account clinical history of antimalarial treatment because of delayed parasite clearance (>1 month) for HRP-2 antigen.[68] In this case, malaria diagnosis should be reached only in the absence of other infectious diseases.

Studies have shown decreasing sensitivity at low parasitaemia. In a study conducted on blood samples of 636 hospital patients in London (non-endemic area) with symptoms suggestive of malaria, recorded 100% sensitivity for ≥500 *P. falciparum* parasites/µl which decreased to ≤73% at lower parasite density (<500 parasites/µl).[25] Highest sensitivity was recorded at ≥5,000 parasites/µl. This is corroborated by study carried out in Port-Harcourt, Nigeria,[64] in Madagascar[69] and on Mount Cameroon region.[70] In that study a pLDH detecting pan-specific

Optimal was used. The sensitivity was also found to be species-related, because in that study, 95.3% was recorded for *Plasmodium falciparum, Plasmodium vivax* (96%), *Plasmodium ovale* (57%) and *Plasmodium malariae* (47%). The last two were found to be quite low. This variation has been reported in mixed infections.[70] The dependence of sensitivity on parasite species was clearly elucidated in previous studies.[64, 65]

In a conversation with O. E. Omede, MD (July 2010), he stated that variance in the test results of RDT specific HRP-II for *P. falciparum* though negative, could be positive with RDT designed to detect mixed infection. This could be due to defective RDT kit and or change in predominant *Plasmodium* species. There are no recent studies that address the change in species-specific prevalence of *Plasmodium* parasites transmission in Nigeria. Despite the factors alluded to above, studies in Enugu and in Ethiopia revealed low sensitivity of for a *P.f* RDT and Parascreen RDT irrespective of parasite density or species variability.[28, 66]

2.6. Proportion of the different *Plasmodium* species in febrile children under the age of 5 years

Plasmodium falciparum infection accounts for 56.9% of fevers in U5 attending public health facilities in Nigeria, a severe form of the disease. [29, 71, 72] In a study carried out among 240 children aged 1-8 years at Rumueme community, Port-Harcourt, Rivers state in 2007, an overall malaria prevalence rate of 27.5% was reported with *P. falciparum* accounting for 25% and other species for 2.5%. *P. vivax* was not found.[69] However, in children 1-4 years old in the same study, a slide microscopy positivity rate of 36.4% and *P. falciparum* infection rate of 100% were found[69]. The same *P. falciparum* infection rate has been reported in studies conducted at the

Federal Medical Centre in Yola, Nigeria[27] and government health centre Ebute-Metta, Lagos[73], Nigeria. In a study conducted in one private and two public health institutions in Owerri from January-March 2005, out of 500 blood samples examined using Giemsa and Leishman stained smears; 99.2% of the identified species were *P. falciparum* while 0.8% were *P. vivax.*[74] The prevalence rate of malaria parasite was 62%.[74] In a multi-centre study which involved children aged 6 months to 5 years in north eastern Nigeria, from 1988-1995, found that the malaria infection rates varied seasonally and are site-specific.[2] *P. falciparum* was the predominant specie constituting at least 90% of infections, *P. malariae* occurred occasionally and usually in mixed infections with *P. falciparum*. *P. ovale* was very rare and appeared in the southern fringes of the zone. *P. falciparum* infection rate was highest in Damboa, Borno state (66%-76%) during the high transmission season and lowest in Kumo, Gombe state (19%) during the low transmission season (April/May). Out of the 6, 285 patients screened at various sites, 3,725 (59.3%) tested positive for malaria[2]. Infection rates were 36.7% in Tafawa/Balewa (Bauchi state) in May/June 1989 and 63.6% in September/October 1989.[2]

However, a higher prevalence has been reported in the same age group. In a study carried out at Ota General Hospital and Covenant University Health Centre,[31] Canaan land, Ota Ogun State in Nigeria reported a prevalence rate of 84.7% with 100% *P. falciparum* infection rate. Regionally, the predominant species is *P. falciparum* in sub-Saharan Africa and is responsible for ≥90% of malaria cases.[13]

2.7. Factors affecting the utilisation of laboratory services in clinical and community settings

It is cheaper to treat malaria presumptively than to do a Giemsa based microscopy before instituting treatment; N660 ($4.49)/child versus N888 ($6.04)/child, as documented in a study carried out in southwestern Nigeria.[75] A study carried out in southeastern Nigeria also showed that it is more cost-effective to treat malaria based on RDT rather than using microscopy results or treating empirically.[57] Cost implication was found to be in this order: microscopy based treatment >presumptive treatment >RDT based treatment. In addition, a study in Gwagalada, Abuja, Nigeria reported that 76.8% of households that had laboratory investigation for malaria in the last month spent N499-N999 ($3.32-6.67).[53] Apart from cost, other factors that limit utilisation of malarial laboratory services include access to health care services. Distance from health centres may also have an influence based on studies in Kenya.[76-79] Access to formal health care in areas of high endemicity is usually low in remote areas and when available, microscopy services are unreliable.[13] A large percentage of Africans (>70%) self-treat "malarial fever" at home with herbal remedies or drugs bought from patent medicine shops and only attend health centres after self-treatment fails, and even so they do not receive a good-quality diagnosis.[80, 81] This propensity for self-medication is poverty-driven.[82]

Free laboratory services and antimalarial drugs, short waiting time for laboratory results, good quality of laboratory services with adequate and correct treatment have been found to be responsible for the high satisfaction rate for malaria laboratory services.[83] On the contrary, "unaffordable fees, long waiting times, unavailability of drugs, and poor attitudes among staff" were reasons why poor people delay presentation at health facility for utilisation of laboratory services.[84]

The perception of patients that the illness is mild, the thought of other diseases, and financial problems, were among factors that discouraged malaria patients from using laboratory services. [83]

Generally, there is dearth of studies on caregivers' perceptions of factors affecting the use of malaria laboratory services. Review of studies on these factors from the perspectives of healthcare provider's perception revealed the following to be associated with non-utilisation of malaria laboratory services: patients' refusal (99%), high cost of services (95%), attitude of physicians (laboratory results having an impact on patient management (44%), doubt of accuracy of test (82%), reliance on laboratory tests to diagnose infection (88%), reliance on clinical judgment to diagnose malaria (64%).[85] However, physicians' reliance on clinical judgment is the most significant barrier to laboratory use. Polage *et al.* (2006) reported 10-15% of malaria diagnosis was supported by positive malaria smear results and this was attributed to "quality of laboratory testing, cultural beliefs of patients, attrition of health care workforce, physicians' attitudes, and scarcity of consumables".[85]

2.8. The capacity of laboratory staff and the laboratory for malaria diagnosis using microscopy in a clinical setting

In a study carried on the assessment of diagnostic capacity of Nigerian public health laboratories in the integrated disease surveillance and response (IDSR), all the 10 labs (5 public health, 5 non-public health) assessed had the basic equipment, reagents, and materials for malarial microscopy but lacked staff with the requisite skills, training, and experience to carry out rapid testing and molecular techniques.[86]

The capacity of a laboratory to deliver effective microscopy services is dependent on workload, staff strength and competence, and availability of laboratory reagents and consumables. In a study on laboratory malaria diagnostic capacity in Oromia, Ethiopia, 24.9% (56/225) of malaria blood films at secondary/tertiary facilities were processed; while 76% (57/75) clinical malaria cases in primary facilities were confirmed using RDTs. [85, 87] In the same study, 24% of the 159 laboratory staff had received malaria microscopy training within the last 12months while 72% of the facilities had at least one functional electric binocular microscope. It is worthy of note, that none of the surveyed laboratory facilities had formal quality assurance/quality control protocols for either microscopy or RDTs.[87] Supervisory or quality-assurance mechanisms, maintenance of equipment are essential for accuracy of malaria microscopic diagnosis.[59]

A published study estimated that a malaria rapid diagnostic test with 95% sensitivity and specificity requiring minimal infrastructure would avert more than 100,000 deaths and about 400 million unnecessary treatments.[88] RDTs have been proven to be of great value, reduced presumptive diagnosis, acceptable by community residents, and have aided extensive hospital and field-based treatment with an excellent end-user performance.[15, 89, 90] The ease of use, availability of RDT of high diagnostic accuracy and its deployment for use on a wide scale will get us closer to achieving the vision of malaria elimination and eventual eradication. However, there is a need to validate malaria RDTs prior to deployment at community level.

This research study sought to establish the diagnostic accuracy of SD Bioline RDT in a clinical setting, determine the parasite based-factors affecting this, evaluate the utilisation of laboratory services by caregivers of under-fives and assess the capacity (skills, training and experience) of

laboratory scientists and the laboratory for malaria services delivery. SD Bioline RDT is one of the three malaria RDTs selected for deployment at health facilities by the National Malaria Control Programme (NMCP); others being First Response and Parachek. This study also aimed at establishing how laboratory results can influence public health policy decisions.

CHAPTER THREE: METHODOLOGY

3.1. Background information on study area(s)

The study was carried out in Makarfi, Kaduna state. The state is located in the north-west geo political zone of Nigeria. It has a total population of 6,827,966 inhabitants distributed in 23 Local Government Areas (LGAs) and 225 political wards. It shares borders with Zamfara, Katsina, and Kano states to the north; Plateau and Bauchi states to the East, Niger state to the west, Nasarawa state and the FCT Abuja to the south. The predominant religion in the population in the northern part of the state is Islam while in the south it is Christianity. Farming and trading are the major occupation of the people. There are over 15 ethnic groups in the state. Kaduna state is located within guinea savannah vegetation belt where malaria transmission is meso-endemic and parasitaemia is season dependent. The peak rainy season is between June and September. The temperature range is 16–35 °C and the annual rainfall is 1000–1500mm. A recent study in Northeastern Nigeria (Maiduguri) has shown a malaria parasite slide positivity rate of about 13.4%, an equivalence of malaria prevalence (Watila IM; MD, unpublished). There are 1150 public health facilities in the state; 1 tertiary facility, 28 general hospitals, 472 primary health care facilities and the rest are health posts. The general hospitals are under the state Ministry of Health while the primary health care facilities are under the Local Government Primary Healthcare Board. Many residents in Kaduna state seek health care at primary and secondary health facilities. Makarfi, a rural and semi-urban LGA; is located in the northeastern part of the state. It has 10 wards, 40 health-posts, 20 primary health care facilities and one secondary health facility (Makarfi General Hospital). The study was carried out at Makarfi general hospital and Makarfi Community in Kaduna state. Makarfi General Hospital, a 51 bed-hospital; was selected because of patronage of services from the surrounding rural and semi-urban communities,

presence of microscopy facilities, and the presence of a laboratory. Average patient load was 400 persons per month of which about 50 were children below 5years.

3.2.1 Study design

The study design was a combination of cross-sectional (utilisation of laboratory services), laboratory study (accuracy), and qualitative study (focus group discussion and key informant interviews) methods.

3.2.2 Study population

All children under 5 years presenting with fever at Makarfi General Hospital during the study period and their parents/guardians, and members of the Makarfi community, Kaduna state.

3.2.3 Eligibility criteria for febrile children

Inclusion criteria: All children below 5years, presenting with fever and temperature ≥37.5°C (axillary body temperature) or history of febrile illness in the last 24hrs, whose parents have consented to participate in the study, were enrolled. Clinical fever was ascertained using a clinical thermometer; otherwise history of fever was used.

Exclusion criteria: Any patient <6months or 5years and above or any child without history of febrile illness and or with fever with temperature <37.5°C and any parent who declines to participate in the study. Patients with fever and rash or any form of bleeding disorder were excluded.

3.2.4 Eligibility criteria for community members

Inclusion criteria: caregivers (mothers, fathers, guardians) of children below 5years who gave informed consent to be interviewed.

Exclusion criteria: Any caregiver (mothers, fathers, guardians) who declined to be interviewed.

3.2.5 Sample size determination

For the study, sample size was estimated using the formula for determining sample size for cross-sectional studies[91].

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

In the absence of a readily available, published study on Makarfi showing the proportion of malaria cases in children less than 5years, a reported a prevalence of 76.4% of malaria in children below 5years at the Federal Medical Centre, Yola, was used to estimate the sample size for the present study [27].

p = Proportion of under 5 children with malaria = 0.764

q = 1-p = 1 - 0.764= 0.236

z = 1.96 = value at 95% confidence coefficient at α = a significance level of 0.05.

d = 0.05 = precision of study

$$= \frac{1.96^2 (0.764 \times 0.236)}{0.05^2}$$

$$= 277$$

A minimum sample size of **277** was used for this study.

Parasite density was determined for all the positive thick smears.

3.2.6 Sampling technique

Febrile children/caregivers: Caregivers of children aged below five years, attending the medical outpatient department of the Makarfi General Hospital were stratified into those presenting with children who have febrile illness and those without. The caregivers (n=300) in the eligible group were selected consecutively and enrolled in the study in line with the eligibility criteria.

Community: Caregiver (mothers, fathers, guardians) of children less than 5years were stratified into males and females. Each group was further stratified into caregivers whose children have ever had fever and a study group of seven each was then selected purposively.

3.2.7 Data collection

The study was carried out from December 2010 to August 2011.

Questionnaire

Standardised, structured, and pre-tested questionnaires were administered to parents of children below five years, who presented to Makarfi General Hospital (MGH) by the trainee epidemiologist (the principal investigator) and a trained interviewer. The following variables were assessed:–

- Demographic variables: child's age, gender, caregiver's level of education, caregiver's occupation, residence (rural or urban), caregiver's marital status, religion, distance from caregiver's house to the hospital;
- Clinical information: onset of symptoms, drug history/medications used, awareness of laboratory diagnostic services for malaria, use of laboratory services during previous episodes, factors determining utilisation of laboratory diagnostic services;
- Laboratory results.

The questionnaire was translated into Hausa language and back-translated into English to avoid any ambiguity, and then pretested at Hajia Gambo Sawaba General Hospital, Zaria, Kaduna state.

Clinical data was collected from 300 eligible study participants using the standardised questionnaires. Four questionnaires were discarded because of incompleteness.

3.2.8 Laboratory methods

SD Bioline (Standard Diagnostics, Kyonggi-do, Korea) Malaria RDT LOT 082070, expiration date 09/08/2012), a two-band Malaria RDT that detects Histidine-Rich Protein II antigen of *Plasmodium falciparum* was used. It is one of the RDTs currently deployed by the NMCP and selected from the list of WHO Prequalified RDT kits. Blood samples of eligible patients were taken by finger prick under aseptic conditions and screened with the SD Bioline RDT kits using microscopy as the gold standard. On the SD Bioline RDT cassette, **positive result was shown when** one red line in window **"C" (Control) and** one red line in window **"T" (Test)** meant the patient **did** have *falciparum* malaria. **For a negative result: one red line** in window **"C"** and **no line** in window **"T"** meant the patient did not have *falciparum* malaria. Absence of a line in window **"C"** meant the test was damaged and result was **invalid**. The results of the RDT were read within 15minutes.

Microscopic examination of the Giemsa-stained blood smear was carried out. Thin and thick blood films were made to identify the different species of *Plasmodium* and assessed parasitaemia from the samples respectively. The results of the blood film were processed daily or every other day depending on workload. The two trained laboratory scientists (each of whom was responsible for handling each test separately and non-staff of MGH were blinded to the results of RDT and microscopy.

Flow chart for diagnostic accuracy component of the study see appendix 3.2.7.1

Parasite density in the thick blood smear was assessed by counting the number of asexual parasites against ≥200 leucocytes in a thick film, and converting the value to parasites/μL using the standard of 8,000 leucocytes/μL. Hand tally-counters were used to count a field to the end. The parasitaemia was calculated according to the formula below.

$$\text{Parasite density (number of parasites/μl)} = \frac{\text{Parasite count x 8,000}}{\text{No of WBC counted}}$$

A blood slide was deemed negative when the examination of 100 thick film fields did not show the presence of asexual forms of *P. falciparum*. In addition, 100 fields of the thick film were examined for the exclusion of mixed infections**.**

Selection criteria for microscopists

Microscopists were selected based on possession of laboratory expertise to identify and differentiate between the stages in the life cycle of the parasites and laboratory skills to speciate malaria parasites into *P. falciparum, P. vivax, P. ovale and P. malariae*.

Case Report Form

Laboratory data was collected using the case report form (CRF) from 296 patients enrolled. However, only 295 results of blood smear examination and malaria rapid tests were considered for laboratory data analysis and interpretation. The slide for one of the result could not be retrieved.

Quality assurance

The malaria rapid test kits were stored at room temperature throughout the period of the study. The tests were carried out by trained and experienced laboratory scientists. Quality assurance for the malaria rapid test kits and slides was carried out at WHO/TDR Malaria Diagnosis Quality

Assurance Collaborating Centre, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria. Quality assurance for the identified microscopists: two microscopists were engaged to ensure accuracy of test results and blinded rechecking was carried out. The microscopists were blinded to the results of RDTs.

Patient management

The SD Bioline RDT and microscopy results were written on patients' hand card and forwarded to the clinician to serve as a guide on the treatment decision. All patients with positive test results were treated as necessary on the same day of visit. Patients with negative results received further assessment and an appropriate treatment strategy given.

Based on findings of reported lack of awareness of caregivers about malaria laboratory services and non-request of these services by health staff, we collected qualitative data at the community level to complement the quantitative data on factors influencing utilisation of malaria laboratory services.

Focus group discussion notes

All the three facilitators (moderator, note-taker, and observer) were trained on the study objectives. Hints were given on the use of appropriate probes and prompts where necessary. The research questions were studied, explored and translated into Hausa for ease of administration. Field visit to the two communities was undertaken by the trainee field epidemiologist to ensure the quality of the data collected. Focus group discussions (FGDs) with residents of Makarfi community were carried out in two communities, namely: Kuruntumawa (a rural community, 45km from Makarfi town) and Makarfi town, to ascertain the factors determining utilisation of laboratory services for malaria diagnosis. The participants were mobilized by the community leaders through the local government development committees and selected purposively-they

had ever cared for a child with fever, below 5 years. Quiet and conducive atmosphere and a semi-circular sitting arrangement were usually ensured before each session to enhance the quality of the recording as well as the relaxation of the participants. Four FGDs with seven participants in each group of the caregiver (2 groups each of men and women) were done at an appropriate place selected by the community leader and with the consent of the participants. The discussion was conducted in Hausa language and recorded on notes and tape recorder. The interviewer then translated the transcribed notes into English. The sessions were immediately transcribed and translated.

Checklist

Using a checklist, a Key Informant Interview of the head of the laboratory and other staff was carried out by the principal investigator, to assess the capacity of laboratory staff and the laboratory for malaria diagnosis. The pre-designed interview guide was pretested at Hajia Gambo Sawaba Specialist Hospital and used to collect information on staff strength and years of experience, their academic qualifications, and level of training on malaria microscopy, workload, availability and utilisation of standard operating procedures, availability and status of equipment, materials and supplies.

3.2.9 Data processing and analysis

Diagnostic accuracy component of the study

- Sensitivity, specificity, positive predictive value and negative predictive values of RDTs were determined

Data entry, cleaning and analysis were done using Epi-info version 3.5.3. A descriptive analysis of demographic and clinical information was carried out. Variables were presented as percentages. Statistical analysis using chi square statistical tests was done to compare categorical

variables where appropriate and considered significant at $\alpha < 0.05$. Tables and figures were used to describe the results.

Qualitative studies:

Focus group discussion

Issues emanating from the FGDs were identified after each session and summarized. Observational notes were written after every interview. All translated FGDs were properly studied. Similar themes were found and categorized. Interpretation of the notes was conducted in narrative.

Key Informant Interview

The health workers' responses to the interview questions were described in narrative.

3.2.10 Ethical consideration

The perceived benefit of the study to the caregivers of children below 5 years was an instant diagnosis of malaria with RDT at no cost. Approval was sought from Kaduna state Ministry of Health. Ethical approval was sought from National Health Research Ethics Committee, Federal Ministry of Health, and Scientific and Ethical Committee of the Ahmadu Bello University Teaching Hospital Zaria.

Oral Informed consent was taken from the caregivers of children less than 5 years presenting with fever. Confidentiality of information given was assured and maintained.

3.2.11 Dissemination plan

The findings from this study will be disseminated to the National Malaria Control Programme and Kaduna State Ministry of Health. The abstract of the study will be available for presentation at scientific conferences. Summary report will be published in peer-reviewed journals.

The study was conducted in compliance with standards for reporting studies of diagnostic accuracy. These standards defined accuracy as "the amount of agreement between the results from the index test and those from the reference standard"[92, 93].

3.2.12 Limitations

This study was carried out during the low transmission season (December, 2010 – May, 2011), partly high transmission season (June – August, 2011) and this might have influenced the prevalence and level of parasitaemia; hence, the RDT results[2]. The results may be more representative if the study is carried out all year round. In addition, only a brand of RDT kit was used. Comparison of diagnostic accuracy of two or more RDT brands would have been ideal. Selection of patients/participants presenting only at Makarfi General Hospital and Makarfi town only and may not reflect the perception of the generality of the populace in Kaduna state. Views expressed by these groups could defer from those of other communities within the same LGA.

CHAPTER FOUR: RESULTS

The results of analysis of 296 caregivers and children below the age of 5years are presented below.

4.1. Socio-Demographic Characteristics

Table 4.1.1: Distribution of Caregivers by Socio-demographic Status, Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Characteristics (n=296)	Frequency (%)
Level of education	
None	207 (69.9)
Informal	8 (2.7)
Primary	37 (12.8)
Secondary	35 (11.8)
Tertiary	8 (2.7)
Occupation	
Civil servant	10 (3.4)
Farmer	65 (22.0)
No formal employment	207 (66.9)
Trader	5 (1.7)
Others	9(3.0)
Marital status	
Married	275 (92.9)
Single	16 (5.4)
Widowed	5 (1.7)
Religion	
Christianity	72 (24.3)
Islam	224 (75.7)

Over two-thirds of the respondents had no formal education (69.9%). More than two-thirds of the respondents were not formally employed (69.9%). Over 90% of the respondents were married. Three-quarters of the caregivers (75.7%) were Muslims.

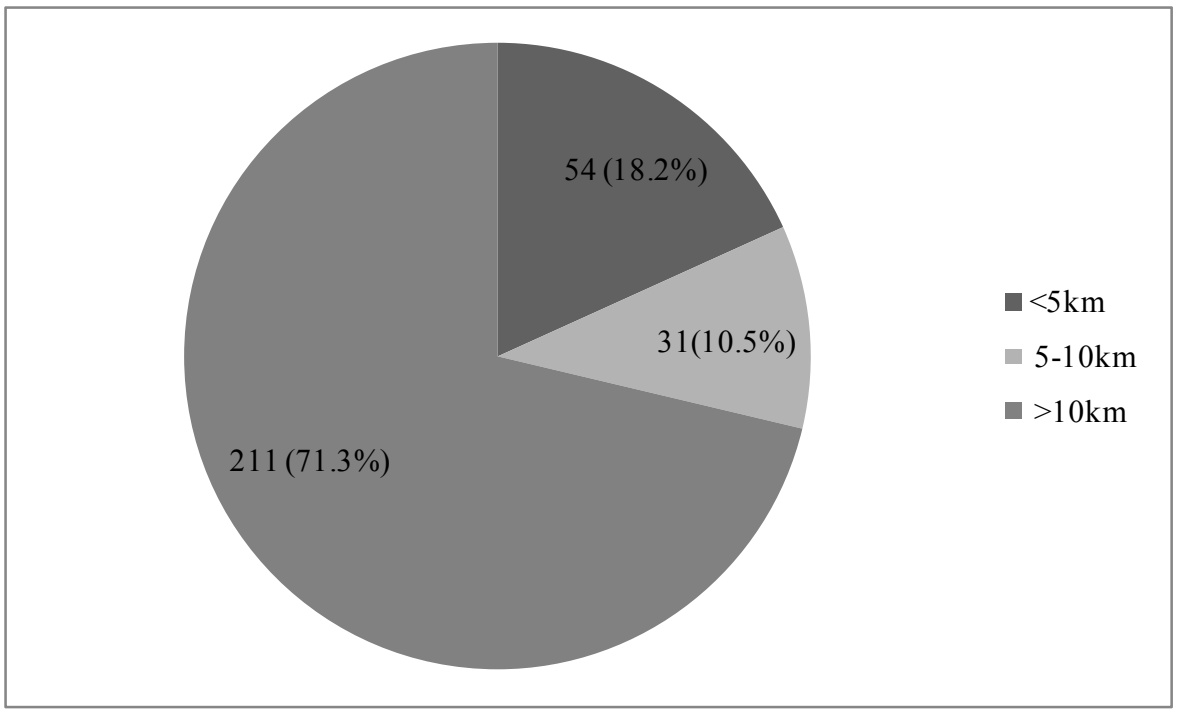


Figure 1: Distribution of Caregivers by Distance of Residence from Makarfi General Hospital, Kaduna State, December 2010 - August 2011

More than four-fifths (81.8%) of the caregivers live beyond 5km walk from Makarfi Hospital.

Table 4.1.2: Socio-Demographic Characteristics of Febrile Children Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Characteristics (n=296)	Frequency (%)
Child's age (months)	
6 - 11	59 (19.9)
12 - 23	62 (20.9)
24 - 35	55 (18.6)
36 - 47	46 (15.5)
48 - 59	74 (25.0)
Child's sex	
Male	163 (55.1)
Female	133 (44.9)

Of the study participants, more than half (55.1%) were males. Median child's age was 24 months (Inter-quartile range 14-47 months).

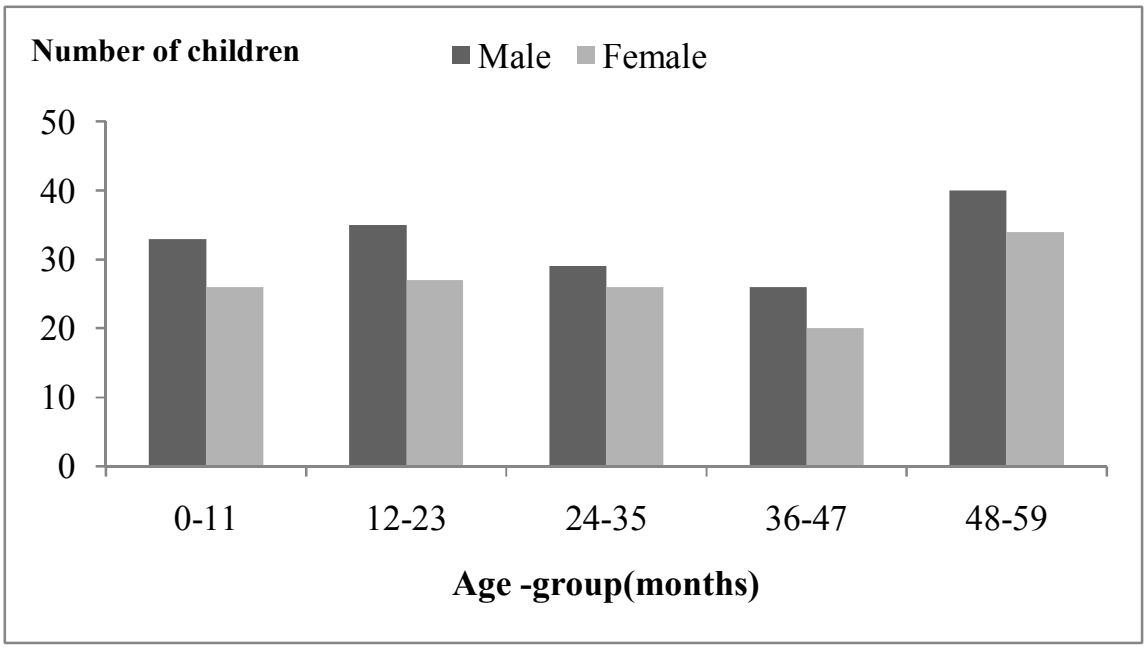


Figure 2: Age and Sex Distribution of Children Less Than 5years Screened for Malaria Parasites at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Males comprised a higher proportion of the febrile children screened for malaria irrespective of age group. Majority of children were ages 48-59months.

4.2: Sensitivity, specificity and predictive values of SD Bioline RDT

Table 4.2.1: Distribution of *P. falciparum* Malaria by Parasitological Technique in Febrile Children at Makarfi General Hospital, Kaduna state, December 2010 - August 2011

	Microscopy		
	Positive	Negative	Total
RDT	Frequency (%)	Frequency (%)	Frequency (%)
Positive	31 (100)	4 (1.5)	35 (11.9)
Negative	0 (0)	260 (98.5)	260 (88.1)
	31 (100)	264 (100)	295 (100)

Sensitivity: 31/(31+0)*100 = 31/31*100 = 100%

Specificity: 260/(4+260)*100 = 98.5%

Positive Predictive Value: 31/(31+4)*100 = 31/35*100 = 88.6%

Negative Predictive Value: 260/(0+260)*100 = 260/260 = 100%

SD Bioline RDT was highly sensitive and specific

4.3: Factors influencing sensitivity, specificity and predictive values of SD Bioline RDT (parasitaemia and species)

Table 4.3.1: Variation of Sensitivity of SD Bioline Malaria Rapid Test By Parasite Density at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Number of Participants Tested with Concordant Results

Density of	**RDT positive (%)**	**Microscopy positive (%)**	**Sensitivity %**	
P. falciparum				Mean Parasitaemia (/µL)
≤100	0 (0)	0 (0)	Not applicable	
101-500	3 (9.7)	3 (9.7)	100%	266
501-1,000	9 (29.0)	9 (29.0)	100%	780
1,001-5,000	8 (25.8)	8 (25.8)	100%	3307
5,001-10,000	4 (12.9)	4 (12.9)	100%	7357
>10,000	7 (22.6)	7 (22.6)	100%	29531
	31 (100)	31 (100)	100%	

The median parasite density in this study was 3, 184 parasites (range: 159 - 57,056)/µL.

The overall mean parasite density, 8,723 ±13,655 parasites/µL.

4.4: Proportion of *Plasmodium* species

Table 4.4.1: Slide and RDT Positivity Rate of Blood Specimens of Children below Five Years Screened For Malaria at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

RDT	Thin film microscopic examination	Thick film microscopic examination	Slide positivity rate (%) (N=295)	RDT positivity rate (%) (N=296)	Species	% of total positive
296	295	295	31 (10.5)	35 (11.8)	*P. falciparum*	100%

The prevalence of malaria was 10.5% and 11.8% by microscopic and RDT respectively. Examination of the blood films showed 100% *Plasmodium falciparum* infection rate.

N.B. Peripheral blood film microscopy result of a patient could not be retrieved.

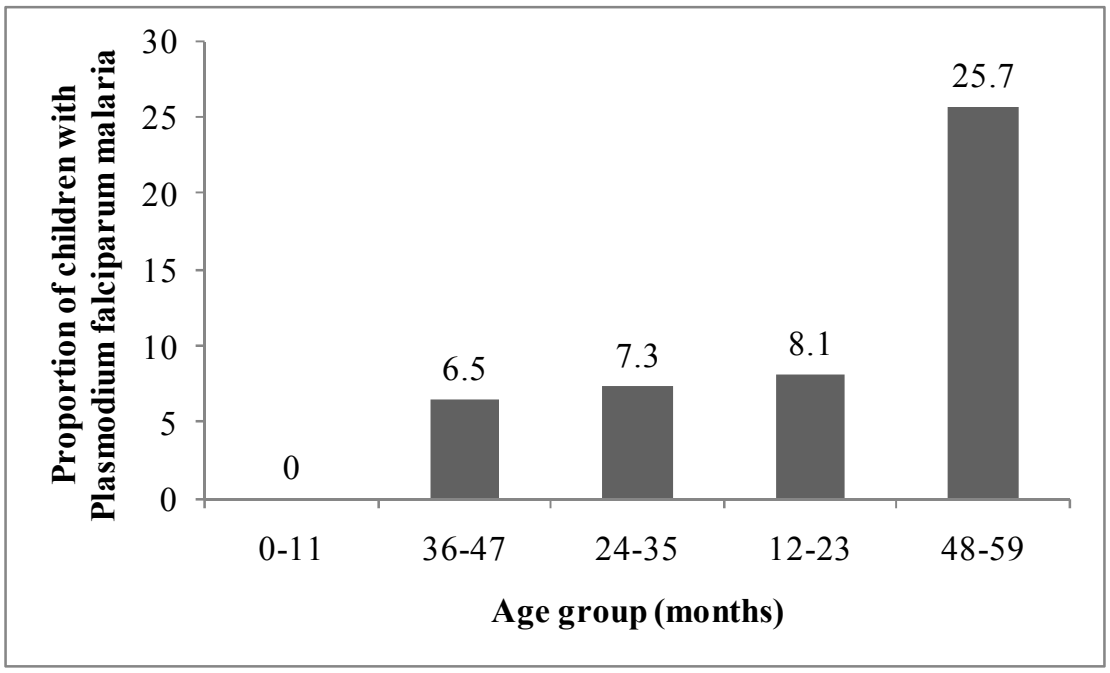


Figure 3: Age-Specific Proportion of Febrile Children Presenting at Makarfi General Hospital, Kaduna State, with *Plasmodium falciparum* Malaria, December 2010 - August 2011

There is an increasing proportion of malaria presence with age. This is three-four times in 48-59months compared to each of the preceding age group except for in infants where no malaria parasites were found.

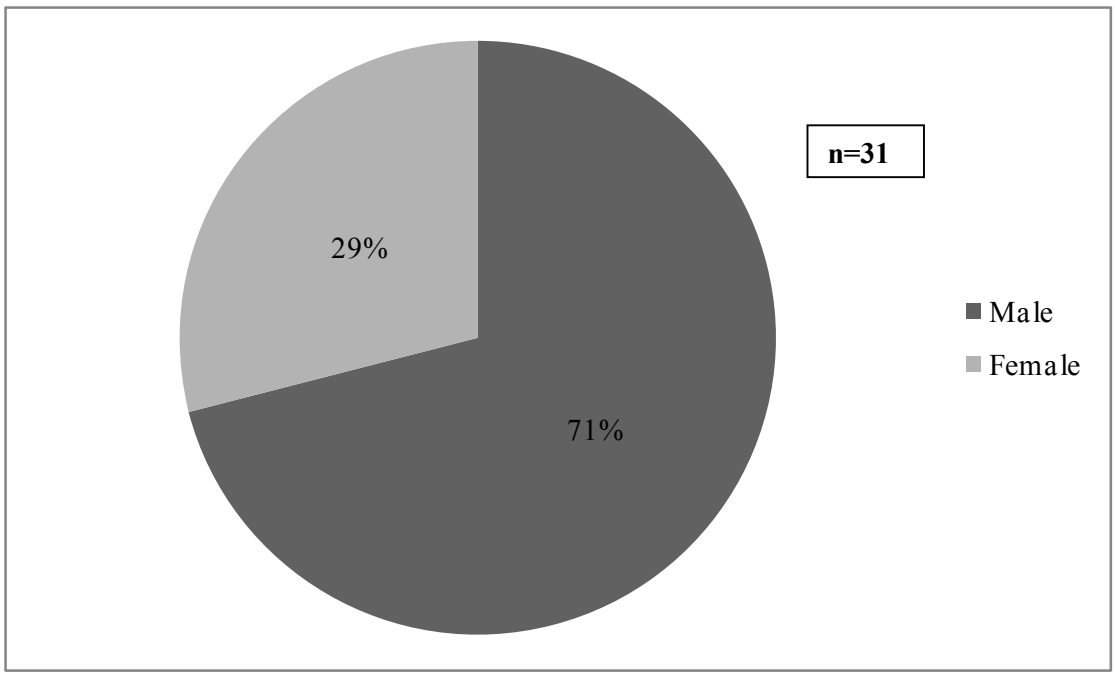


Figure 4: Sex Distribution of *Plasmodium falciparum* in Positive Blood Smears of Febrile Children Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

There were 31 positive cases of malaria, all of which 22 (71%) and 9 (29.0%) were males and females respectively.

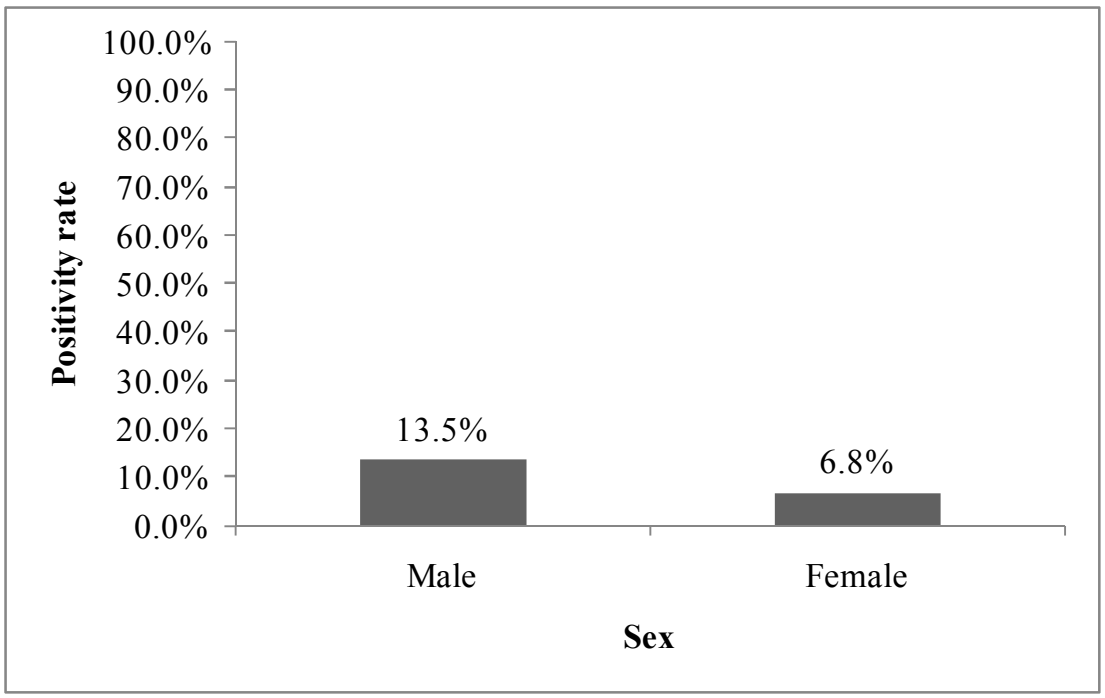


Figure 5: Sex Distribution of Slide Positivity Rate of Malaria in the Febrile Children Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

The positivity rate of malaria was found to be twice in male compared to females.

Table 4.4.2: Distribution of Clinical Signs and Symptoms By Rapid Diagnostic and Parasitological Techniques of Children Below Five Year Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Symptoms/Signs	RDT positive (%) n=35	Microscopy positive (%) n=31
	No (%)	No (%)
Fever	29 (82.9)	26 (83.9)
History of fever	33 (94.2)	28 (90.3)
Headache	8 (22.9)	7 (22.6)
Cough	17 (48.6)	13 (41.9)
Catarrh	5 (14.3)	1 (3.2)
Chills and /Shivering	21 (60.0)	18 (58.1)
Vomiting	14 (40.0)	14 (45.2)
Diarrhoea	12 (34.3)	9 (29.0)
Nausea	1 (2.9)	1 (3.2)

Among the children who were diagnosed with malaria by RDT or microscopy, over 80% and 90% had clinical fever and history of fever respectively.

Table 4.4.3: Clinical Presentation of Febrile Children at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Signs/Symptoms	Number of patients (%) n=296
Fever	272 (91.9)
History of fever (n=283)	277 (97.9)
Headache (n=295)	67 (22.7)
Cough	206 (69.6)
Catarrh	90 (30.5)
Chills and rigours	103 (34.8)
Joint/body pains/weakness	37 (12.5)
Vomiting	68 (23)
Diarrhoea	106 (35.8)
Nausea	6 (2.0)
Abdominal pain	165 (55.7)
Bitter taste	8 (2.7)

Over two-thirds (69.6%) of the symptomatic febrile patients, presented with cough. Of the 296 respondents, 272 (91.9%) had clinical fever (verified by thermometer ≥37.5%). Of the 283 who answered the question on history of fever, 277 (97.9%) had only history fever. Median duration of symptoms was 3days (Inter-quartile range: 1-6 days)

Table 4.4.4: History of Pre-Hospital Treatment of Febrile Children Who Presented at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Characteristics	Number of patients (%)
Received any form of treatment	201 (67.9)
Kind of treatment administered (n=201)	
Oral drugs	176 (87.6)
Oral and injectables	18 (9.0)
Herbal	11 (5.5)
Oral and Herbal preparations	40 (19.9)
Oral, herbal and injectables	7 (3.5)
Kinds of oral drugs (n=176)	
ACT	1 (0.6)
Chloroquine	24 (13.6)
Antibiotics	32 (18.2)
Antipyretics	30 (17.0)
Time of administration of pre-hospital treatment(n=201)	
Same day	20 (10.0)
Next day	33 (16.4)
3days after	26 (12.9)
≥4days after	78 (38.8)

Over two-thirds of (67.9%) children had received a form of pre-hospital treatment with 53(26.4%) receiving this within the first 24hrs of onset of symptoms. Of the pre-hospital treated children, 87.6% and 5.5% were treated with oral drugs and herbal medication respectively. Antibiotics and ACT were the most and least used drug respectively. Median time of administration of pre-hospital treatment was 3 days (Inter-quartile range: 2-5 days).

Table 4.4.5: Previous Fever Episodes and Treatment-Seeking in the Last 1year for Any of the Children of the Caregiver Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Characteristic (n=283)	Number of children	%
Previous episodes of fever		
1	52	18.4
2-4	72	25.4
≥5	8	2.8
Can't remember	69	24.4
Don't know	77	27.2
Sought medical care	232	82.0

Two hundred and eighty-three caregivers (95.6%) responded to whether they had any child/children with at least an episode of fever in the preceding 12months. Of these, 72 (25.4%) had children with 2-4 episodes. Of the 283 caregivers, 232 (82%) sought medical care.

Table 4.4.6: Point of Treatment in the Last 1 year for Any of the Febrile Children of the Caregiver Presenting at Makarfi General Hospital, Kaduna State, December 2010 - August 2011

Characteristic	Number of children	%
Site of medical care (n=232)		
Primary Health Care centre (PHC)	51	22.0
General Hospital	22	9.5
Private health facility	63	27.2
Patent Medicine Store	121	52.2
Herbalist	38	16.4
Primary health care centre and Herbalist	1	0.4
General Hospital and Private health facility	1	0.4
General Hospital and Herbalist	1	0.4
Patent Medicine Store and General Hospital	4	1.7
Patent Medicine Store and PHC	19	8.2
Patent Medicine Store and Herbalist	19	8.2
Patent Medicine Store and Private health facility	9	3.9
Private health facility and Herbalist	2	0.9
PHC, General Hospital and Herbalist	1	0.4
PHC, Patent Medicine Store and Herbalist	1	0.4
Patent Medicine Store, Private health facility and Herbalist	4	1.7

Of the 232 caregivers, 136(58.6%); 121(52.2%) and 38(16.4%) sought care in a health facility, patent medicine store and herbal home respectively.

Table 4.4.7: Bivariate Analysis of Socio-Demographic Characteristics and Distribution of Malaria in the Febrile Children Presenting at Makarfi General Hospital, Kaduna state, December 2010 - August 2011

Characteristic n=296		**Number positive by microscopy n=31 (%)**	**Number negative by microscopy n= 265 (%)**	**Chi square**	**P value**
Sex	Male	22 (71)	141 (52.3)	2.857	0.091
	Female	9 (29)	124 (46.8)		
Age group(months)	6-11	0 (0)	59 (22.2)		
	12-23	5 (16.1)	57 (21.5)	**7.669**	**0.006**
	24-35	4 (12.9)	51 (19.2)		
	36-47	**3 (9.7)**	43 (16.2)		
	48-59	**19 (61.3)**	55 (20.8)		
Marital status	Married	31 (100)	244 (92.1)	NA	0.1795*
	Single	0 (0)	21 (7.9)		
Religion	Christianity	6 (19.4)	66 (24.9)	0.2119	0.6453
	Islam	25 (80.6)	199 (75.1)		
Residence	<5km	4 (12.9)	50 (18.9)	0.3225	0.5701
	>5km	27 (87.1)	215 (81.1)		

N.B. (NA: Not Applicable, *Fischer's exact test).

Children aged 36-59 months were more likely to have to be diagnosed with malaria compared to aged 6-35 months. Gender differences, marital status, religion, distance of residence from the hospital had insignificant statistical relationship with positivity of malaria laboratory results.

4.5: Factors affecting the utilisation of malaria laboratory services

Table 4.5.1: Awareness of Laboratory Diagnostic Services for Malaria by Caregivers of Febrile Children Who Presented at Makarfi General Hospital, Kaduna State, December, 2010 - August 2011

Characteristics (N=296)	Number of caregivers	(%)
Heard about any lab test	15	5.1
Heard about		
Microscopy	11	3.7
RDT	4	1.4

Only 5.1% of the caregivers have ever heard about a laboratory test for malaria diagnosis.

Table 4.5.2: Utilisation, cost, turnaround time and outcome of Laboratory Services by Caregivers of Children Below 5years Screened for Malaria at Makarfi, General Hospital, Kaduna State, December 2010 - August 2011

Characteristic	Number of caregivers	%
Health worker ever requested for laboratory test (n=273)		
Yes	11	4.0
No	237	86.8
Don't know	12	4.4
Ever accepted to do a malaria laboratory test (n=273)	11	4.0
Cost of laboratory test (n=273)		
Nothing	4	1.5
N200 – 500	4	1.5
N500 – 1000	2	0.7
>N1000	1	0.4
Laboratory turnaround time (n=11)		
Same day	7	63.6
2 – 3days	4	36.4
Outcome of laboratory testing (n=11)		
Positive	4	36.4
Negative	4	36.4
Don't know	3	27.2

Only 4.0% of the caregivers have ever been requested by a health staff to do a laboratory test for malaria. All the 11 (4.0%) agreed to do the test. Six (54.5%) of 11 were placed on ACT.

Table 4.5.3: Distribution of Focus Group Discussants by Sex, Educational Status, Number of Children below 5years

S/N	Name of community	Category of community (18-35yrs)	Educational status	Sex (n)	
				Male	Female
1	Makarfi	Semi-urban	Nil		7
			Islamiyya	2	
			Primary school	2	
			Secondary school	3	
2	Kuruntumawa	Rural	Islamiyya	5	7
			Primary school	1	
			Secondary school	1	

Focus Group Discussion

Common medical problems in Makarfi and Kuruntumawa communities

Majority of the participants said the common medical problems associated with fever in the community were cough, meningitis, typhoid, and malaria. Malaria tends to affect a significant number of children.

The malaria burden

According to a participant, "*Malaria affects both children and adults; about 70% of people*" (female FGD, 18yrs, petty trader, rural). Also, "*yes, that is true, if it wasn't for the nets given to women and children during campaigns, it would have been worse; may be 80% - 90% of people*"

opined, another participant (male FGD, 23yrs, farmer, rural). This was alluded to by another participant, who agreed that "*malaria affects almost all the people in this community both adult and children*" (male FGD, 30yrs, farmer, rural). In the semi-urban community, majority of participants responded that "*80% of children are affected by malaria*" (male FGD, 26-36yrs).

Local names for malaria

One of the respondent submitted, "*Malaria means sickness or illness caused as a result of mosquito bite (Zazzabin cizon sauro)*" (23yrs, FGD male, teacher, rural). Majority of the discussants concurred with this view (female FGD, 18yrs, petty trader, rural).

Accessibility to malaria treatment services

Treatment is sought at home, patent medicine stores, clinics and hospitals. Below are the quotations from the respondents. "*Home treatment first, before going to the hospital*" (female FGD, 25yrs, housewife, semi-urban). "*People used to visit the chemist in the village for treatment, or Meyere village because they have many health personnel*" (male FGD, 23yrs, farmer, rural). According to the male FGD, rural group, "*They normally go to Makarfi General Hospital or Miyari Clinic*" (female FGD, 35years, housewife, semi-urban). "*We normally go to private clinics for a malaria blood test*." "*Some people go to any drug shop and buy Panadol tablets etc*.**"** (male FGD, 30yrs, farmer, rural.

Perception of the need for laboratory services

The perception of the discussants towards the need for malaria laboratory services was varied. Most of the respondents felt the need for laboratory diagnosis is based on severity of the illness. As expressed by a respondent "*In serious cases e.g. Malaria, HIV/AIDS*", according to (male, FGD, 30yrs, farmer, rural). The perspective of the others is similar. "*No, it's not done, blood test is done only when the sickness affects blood, not for malaria*" (male FGD, 30yrs, farmer, rural).

"*Yes, it's true, blood test is done only when patients present with severe illness and loss of weight*" (male FGD, 23yrs, farmer, rural). All in the rural group expressed, "*blood test is done if the condition is critical*" (male FGD, farmer, rural). Misconception was also confirmed by the view expressed by a respondent, "*It is only for blood transfusion*" (male FGD, 25yrs, business owner, semi-urban).

Awareness of malaria laboratory services

There were mixed feelings about the level of awareness of malaria laboratory services. Generally, awareness is low. This is exemplified by the quotations below. "*I once took my child to hospital and was asked to do blood test*" (female FGD, 25yrs, housewife, semi-urban). One of the respondents confessed that he was unaware of malaria and its signs, and therefore blindly administered the medicine given by the doctor (male FGD, semi-urban). Another added: "*We are not aware, they are not aware, all the respondents have not been told about the laboratory service*" (female FGD, 18yrs, petty trader, rural). However, a few of them claimed they heard learnt about it from their friends who visited the hospital. "*Yes, we are aware, through discussion with our friends and visit to hospital*" (male FGD, semi-urban). "*Only two respondents knew about using a blood test to diagnose malaria*" (female FGD, 25yrs, housewife, semi-urban).

Factors affecting utilisation of laboratory services

Utilisation of laboratory services was found to be influenced by presumptive treatment (at home and in the hospital without support from laboratory diagnosis) and the availability of the services within the community. Majority of the respondents claimed they were not offered a laboratory test whenever they visited the hospital. Four out of seven respondents said that "*no blood test was done before that*" (male FGD, semi-urban). According to male FGD, 36yrs, farmer, semi-urban, when asked whether blood test is done before being given drugs, he said "*No*". The entire

respondents agreed (male FGD, semi-urban). This is similar to the response in the female group "*No, when we present our complaints they give us drugs and thereafter dismiss us*" (female FGD, 35yrs housewife, semi-urban). "*I was never asked to do blood test for malaria*" (female FGD, 25 yrs, housewife, semi-urban). One of the respondents submitted that sometimes, when a blood test is offered, the caregivers are not aware of its essence, "*Yes, they ask us to test but we don't know if it's for malaria*" (male FGD, 28yrs, farmer).

Availability of malaria laboratory services determines its usage. According to a respondent who said the people do not use them because "*the services are not available in the community*" (male FGD, 27yrs, farmer, semi-urban). Similarly, "*Because it is not available in the community*" (male FGD, 30yrs, farmer, rural).

In addition to availability of services, requests by healthcare providers; other factors influencing the use of laboratory services for malaria are: cost of services and distance from the health centre. Below are quotations from the semi-urban male FGD. "*If the doctor asks us to do a test, we have to do it; but only when the services are available and affordable*". "*One will only take the test if they have money to buy drugs and to pay for the test, you will do it*". Additionally, the high cost of services greatly affects the ability of caregivers to use laboratory services. According to one respondent, (female FGD, 18years, tailor, semi-urban) "*after taking the blood test and you are diagnosed with malaria or typhoid, at a private clinic, you will have to pay N500 for the test*". The female group of respondents agreed that consent to do laboratory tests for malaria depended on the doctor's request and cost of services. "*If the services are costly and the doctor does not ask me to take the test, I will not*" (female FGD, semi-urban). Findings from the rural communities are similar. In addition to availability and affordability of services, as depicted in the quotes below, proximity to services is a significant determinant. "*If the services are*

available and patients are asked to take the test, they will" (male FGD, 23 yrs, farmer, rural). "A patient will also only take the test if he can afford it" (male FGD, 30yrs, farmer, rural). Conventionally, healthcare providers initiate the request for laboratory services. This is buttressed by the rural female FGD group. The three cardinal reasons influencing access to laboratory services are: availability, affordability, and requests by healthcare providers. One respondent summed this up thus: "*If one has the means i.e. money to pay for the services and the services are available, plus the doctor asked the person to take the test, then they will*" (female FGD, rural).

4.6: Capacity of laboratory staff for malaria diagnosis

Key Informant Interview

This revealed eight laboratory (lab) staff, three lab scientists, two lab technicians and three assistants. Of these, six had ≥5years of experience and the two had 4-5years experience. The age range was 20-45years. Two of the staff were trained on malaria microscopy using strip method as part of the undergraduate curriculum; none has received any training on malaria microscopy. The average request for malaria microcopy was 3-5per day and 6-10slides/day were made. Standard operating procedures were available and were being utilised. Control slides were sighted. The two binocular microscopes were powered by generator and electricity. The basic equipment and materials were available but there was no Giemsa stain. There was no quality assurance mechanism in place for malaria microscopy. The lab does not participate in any external quality assurance. The laboratory had no malaria RDT kit and none of the staff had received any training for such.

CHAPTER FIVE: DISCUSSION

This study was one of the few studies in Nigeria, to examine the diagnostic accuracy of malaria RDT in febrile children below five years, awareness and utilisation of laboratory services for malaria diagnosis, and capacity for malaria diagnosis in the same study. Using microscopy as gold standard, it revealed a sensitivity of 100%, specificity of 98.5%, negative predictive value (NPV) of 100% and positive predictive value (PPV) of 88.6% for SD Bioline HRP-2 Malaria RDT. Peripheral blood film microscopy showed *Plasmodium falciparum* was the only species found in positive slides, which further buttressed its sensitivity. This sensitivity meets the WHO recommendation of ≥ 95% and is consistent with findings from other studies conducted in Ebute-Metta Lagos-Nigeria with a sensitivity of 100% for Immunochromatographic test.[13, 23, 73] Previous studies conducted using *P. falciparum* only RDTs in north-eastern Tanzania[8] and in Uganda, showed sensitivities of 95.4%, 97.2% and 97.6% for Parachek, Parachek Pf and ParaHIT *f*, respectively.[68] However, the result of this study is at variance with other studies which showed lower sensitivities. Studies conducted in Yola, Enugu, Port-Harcourt; Nigeria[27] and in Ethiopia found a sensitivity of 69.7% for Global device RDT, [28] 42.3% for a *P.f* RDT, 47% for SD Bioline RDT *Pf/Pv* [66] and 47.5% for Parascreen, an HRP -2 and pLDH based-RDT respectively.[69] These have been ascribed to decreasing sensitivity with reduction in parasite density and thus, patients with low density malaria parasites are missed.

The specificity of RDT obtained in this study (98.5%) is consistent with that in a study conducted in Ethiopia[28] (98.5%), but lower than 100% for global device RDT assessed in Yola-Nigeria.[27] It is higher than results of similar studies conducted in north-eastern Tanzania and in Uganda which showed 95.9%, 88.8%, and 87.7% for Parachek, Parachek Pf and ParaHIT *f*.[8, 68]

However, it is at variance with findings (42.9% for SD Bioline) in Obafemi-Owode area of Ogun state, Nigeria.[94] Negative predictive value (100%) in this study is consistent with study in Ebute-Metta Lagos-Nigeria,[73] and higher than that (99.3%) reported in north-eastern Tanzania.[8] Lower NPVs of 62.9% and 68% respectively have been reported other in studies conducted in Nigeria.[27, 94] Positive predictive value (88.6%) in this study is at variance with findings of 68% and 77% in other studies.[8, 94]

The high sensitivity recorded in this study is probably due to parasitaemia above the threshold of 100/µL recommended by WHO. This implies that this RDT would be useful in diagnosing all malaria fevers at the community level where there exist no robust laboratory capacity for such. Moreover, being highly specific; SD Bioline RDT can detect the absence of malaria and thus prevent wastage of ACTs. This may persuade health care workers to trust RDT results and reduce dispensing of ACTs for cases not diagnosed as malaria. This highly sensitive and specific RDT could be useful during a low transmission season in an endemic setting because of its perceived cost-saving in prioritizing who to treat amongst febrile cases of children at health facilities.

In this study, the slide and RDT positivity rate for malaria were 10.5% and 11.8% respectively. These are lower compared to findings by of 76.3%, of 76.4%, and of 84.7% in the same age group at Maiduguri[38], Yola[27], and Ota[31] respectively in Nigeria. This could be because the present study was largely carried out in the low transmission season. Moreover, the SD Bioline RDT positivity rate was 11.2% and 9.4% in high and low transmission season respectively. Despite the fact that this apparent seasonal disparity in positivity rate has been supported by

another study carried out in children below 8years at Maiduguri (Samdi *et al.* 2005),[38] the seasonal positivity rate of 10.3% by RDT is low compared with findings of other studies highlighted earlier.[38,27,31] This means malaria is not as common as earlier presumed. Thus, indiscriminate use of antimalarials should be avoided and there seems to be no place for presumptive treatment of febrile illnesses as malaria given the revealed specificity of the RDT. A similar conclusion was reached in a study in rural and urban Zambia.[95] This study showed a higher malaria positivity rate with RDTs than with microscopy. This is expected because false positives are possible with RDTs. Moreover, this study showed SD Bioline RDT has a high diagnostic accuracy in a low transmission season where lower levels of parasitaemia are expected and could be of value in field settings where conditions are often less than optimal.[28] The slide positivity rate was found to be twice in males compared to females. However, there is a statistically insignificant relationship between gender and prevalence of malaria unlike findings from other studies in clinical settings among persons below 28years and children below 8years in Yola[27] and Maiduguri respectively, in north-eastern Nigeria.[38]

The mean parasite density, 8,723 ±13,655 parasites/μL seen in this study is similar to findings of >8000 parasites/μL) [73] among persons below 28years. However, it was far higher than that of 900 parasites/μL,[31] which was found in a study carried out among children below 5years in a higher transmission season in south-west Nigeria. Parasite density is dependent on transmission season[31]. The lowest parasite density recorded in this study (159 parasites/μL), is uncommon in the study environment but for the low transmission season[96]. Variation in parasite density had no effect on the sensitivity of the RDT, as this was constant. This is contrary to findings by Moody *et al.* (2000) in non-immune visitors to Asia, sub-Saharan Africa and South America; where

11.7% (48 of the 409 positive thick smear) had parasite density of <100/µL,[25] and Ratsimbasoa *et al*. (2008) in Madagascar who found 19.2% (21 out of 109 positive thick smear) .[64] This could also be explained by the fact that, the parasite density was consistently above 100 parasites/µL, a possibility in a typical endemic setting.

Respective studies in Madagascar, [64] Port-Harcourt-Nigeria,[69] Cameroon,[70] showed that species of *Plasmodium* can affect the sensitivity of malaria RDT but was not the case in this study as all the positive cases on blood smear were *Plasmodium falciparum* i.e. 100% *P.f* infection rate. This is at variance with the findings of national malaria indicator survey[97] among children below 5years which revealed a national prevalence of 95% for *Plasmodium falciparum,* 6% for *Plasmodium ovale* and 10% for *Plasmodium malariae* and 10% for mixed infections. A specificity of 100% is expected for an HRP-2 based RDT but for the false positive results recorded. The false positive results could not have resulted from other non-*falciparum* malaria because there was no record of such in this study. But, this could be explained by presence of dead parasites as a result of pre-hospital treatment with antimalarials.[68, 73] In addition to this, RDT false positivity can be due to detection of gametocytes in the absence of asexual forms, "detection of low-density microscopy-negative infections, or presence of antigenemia early in infection before parasites are detected by microscopy".[98] It can also be due to detection of persistent HRP-2 antigenemia despite parasite clearance. [68] This precludes the use of this RDT to monitor response to treatment in cases of *P. falciparum malaria* cases.[68] However, it would be of value to carry out the same study using combined non-HRP-2/HRP-2 based-RDT.

Only *Plasmodium falciparum* was found in children below 5years whose blood was examined. This is consistent with findings from a study in Yola among persons below 50years.[27] The fact that only *P. falciparum* was diagnosed, has implication for the choice of RDT for mass deployment to health facilities in the country as well as management of cases especially for severe disease.[29, 99] Evidently in the study area, *P. falciparum* infection rate implies that HRP-2 RDT is more valuable for accurate diagnosis compared to LDH-based/other non-HRP-2 based RDT. This is consistent with findings from another study in Yola, Nigeria.[27] Additional evidence comes from other studies in Ebute-Metta, Lagos; Nigeria among persons aged 1-28years and Ota, Ogun state-Nigeria among 0-12years old children (Mosanya and Odujoko 2008; Olasehinde et al., 2010).[31, 73]

Results from the health facility arm of the study showed a positive relationship between having heard about blood test for detecting malaria (15 of 296, 5.1%) and ever being requested by a health worker (11 of 273, 4%) to do a laboratory test for malaria ($p<0.001$). Also, evidence from the Makarfi community supports this lack of awareness of MLS. This could influence utilisation of malaria laboratory service. In the preceding 12 months, sites of treatment-seeking (n=232) for those who sought any form of care for their febrile children) were in this order: healthcare centres (58.6%), patent medicine vendors (52.2%) and herbal homes (16.4%). Treatment-seeking in herbal homes is lower than recorded (25.5%) by a study carried out among mothers of children below five years attending primary health care centres in Ibadan, Nigeria.[80] This is consistent with the reported findings that fewer people still access health centres for treatment of febrile illness. This is a source of concern for the ACTs which are often prescribed at these centres in rural settings.

Access to health services is determined by availability of services; cost of services and geographical location to the population it serves.[100] The distance to the nearest primary health care centre is ≤5km or within 30minutes walk from residence and has been used as a measure of accessibility to laboratory services.[101, 102] In this study, more than four-fifths of the respondents live in areas >5km from a health facility. Interview of caregivers presenting at the Makarfi General hospital showed all those (n=11) who were offered malaria laboratory services accepted to do the test irrespective of the cost. However, this was not the case at the Makarfi community, where non-utilisation of health care facilities and MLS was attributed to high cost of these services, non-availability of services and long distance from point of service. These findings have been corroborated by studies conducted in Ghana and in rural Kenya.[13, 76- 78, 85, 79, 100] According to a study in rural setting of Southern Ethiopia[103] found walking distance of ≥1hr to the health facility discouraged early visit to and treatment initiation at health facilities. This lack of access in addition to high costs, inefficient and suboptimal health care delivery was found to promote self treatment as opposed to health facility-based treatment.[104] The aforementioned factors can be taken into account in improving access to affordable malaria diagnosis in the community. The provision of affordable antimalarial treatment and malaria diagnosis will help reduce morbidity and mortality from malaria as Nigeria strives to achieve millennium development goal six.[105]

Furthermore, non-request for laboratory diagnosis by healthcare providers was found to be a deterrent to utilisation of MLS. Over-reliance on clinical judgement is the single most important barrier to accessing laboratory services.[85] A study on utilisation of laboratory services by health

workers in Malawi showed under-requesting of commonly available laboratory tests.[106] This prevailing attitude could be responsible for self-medication at home which respondents often resulted to, for febrile illnesses. However, the presence of an accurate reliable malaria RDT and adequate supportive supervision is likely to avail a paradigm shift in the diagnostic behaviour of clinicians from presumptive treatment to evidence-based treatment.[107]

According to findings of this study, the presumptive treatment was often sought at the patent medicine stores without prior confirmation of diagnosis contrary to the national guidelines.[108] This is contrary to the national policy on malaria diagnosis and treatment which recommends parasite-based diagnosis in all age groups. And it portends serious consequences for sustained efficacy of ACTs - the only currently effective antimalarial.[62, 95, 108] Resistance to ACTs is imminent as evident in eastern Myanmar.[61] In the same vein, this study revealed caregiver's perceived severity of illness has an influence on seeking of health services including MLS in the formal health sector. This is supported by findings from a study in rural Ethiopia.[83]

Insufficiency of healthcare workforce as a barrier to laboratory testing was found in a study conducted in Ghana.[85] On the contrary, this study revealed an adequate number of laboratory staff with some years of experience. However, they lacked formal training for peripheral blood microscopy. This lack of experienced and trained laboratory scientists is likely to discourage the utilisation of MLS. Formal training is crucial to the delivery of quality laboratory results though the staff might be insufficient for the services rendered.[67, 87] This in turn, is responsible for high satisfaction rate of patients towards MLS.[83] Though equipment and consumables were in place, the laboratory workload for malaria microscopy found was minimal and basic laboratory

reagents were unavailable. These factors are likely to contribute to lack of skilled laboratory personnel to offer MLS as skills acquisition is dependent on hands-on experience and number of slides examined; i.e. work-exposure.[67] This is buttressed by other studies in Ghana, [85] Nigeria[86] and Ethiopia,[87] respectively; which found that capacity of a laboratory to deliver effective microscopy services is dependent on workload, staff competency and availability of laboratory consumables, equipment and reagents. The lack of capacity for malaria diagnosis found in this study is similar to findings of an assessment of 10 laboratories across Nigeria.[86] This portends a grave danger for the quality of laboratory services for confirmatory malaria diagnosis and will adversely affect the implementation of the recommendation of national policy for parasite-based diagnosis for malaria in all age groups.[108] There was no internal quality assurance mechanism in place for malaria nor was the lab participating in any external quality assurance for either microscopy or RDTs. A study in Oromia Region, Ethiopia found similar results.[87] The importance of effective quality assurance mechanisms for accuracy of malaria microscopic diagnosis has been underscored. [59]

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1: Conclusion

The SD Bioline RDT was highly sensitive and specific, and it had high predictive values. The patients who had and who did not have malaria were correctly detected.

The sensitivity was stable irrespective of levels of parasitaemia, and parasite prevalence. The parasite density was found to be higher than 100parasites/μL, which is the threshold for the test. The diagnostic accuracy of SD Bioline RDT remained high in a mesoendemic, low malaria prevalence and parasite density setting.

Plasmodium falciparum was the only species detected in the blood smear of febrile children below the age of 5years found to have malaria in this study. This species remained prevalent after exclusion of other species with expert blood smear microscopy.

Over-reliance on clinical judgment by health staff, lack of awareness of MLS, self-treatment at home, treatment-seeking at patent medicine stores, long distances from point of service, caregiver's perceived severity of illness, high costs and non-availability of malaria laboratory services were some factors responsible for poor utilisation of malaria laboratory services in caregivers of febrile children attending Makarfi General Hospital and members of Makarfi community

There was inadequate capacity for malaria diagnosis. The laboratory staff at Makarfi General Hospital lacked the relevant experience and skills to carry out malaria diagnosis. None of the

staff had undergone formal training on malaria microscopy and RDT. Basic laboratory reagents for malaria microscopy were not available. There was no quality assurance mechanism in place for malaria microscopy and RDT.

6.2: Recommendations

Based on the findings in this study,

1. Kaduna state and local governments, through the state ministry of health and primary health care board should ensure rapid deployment of malaria SD Bioline HRP-2 RDT to health facilities in areas without access to microscopy/where big laboratories cannot be situated. Its deployment for use at the community level and patent medicine stores should be encouraged.
2. The Director of Medical and Laboratory Services, Kaduna state should sensitise health workers and provide them with information, education, and communication materials on the importance of laboratory confirmation of malaria diagnosis in febrile children. Other avenues, such as mandatory training on parasite-based malaria diagnosis before renewal of medical practicing license could be utilised.
3. The malaria unit at Makarfi local government area should sensitise caregivers in the rural/semi-urban communities in Makarfi communities on the need to use malaria laboratory services for their febrile children below five years rather than treating presumptively at home or using patent medicines. This unit should create awareness about these services through community outreaches to various community groups e.g. women groups.
4. The Kaduna state government should provide affordable access to malaria laboratory services in rural communities. The already existing community-based interventions e.g. home management of malaria (in World Bank-supported states) as a vehicle for the delivery of malaria diagnostic and treatment services should be used.

5. Kaduna state and local governments should, as a matter of urgency, strengthen malaria laboratory services in health facilities in rural areas through provision of basic laboratory reagents, equipment and materials, supervisory and set-up of quality assurance mechanisms, and ensure adequate and proper training of laboratory staff on malaria diagnostic services.

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Appendix 1: Patient Information and Consent Form

Assessment of diagnostic accuracy of Malaria rapid diagnostic test and utilisation of laboratory services for Malaria diagnosis at Makarfi general hospital, Kaduna state

Good morning/afternoon/evening. My name is _____ and I am working with Nigerian Field Epidemiology and Laboratory Training Programme. I am a master of Public Health student at Ahmadu Bello University, Zaria. We are conducting an assessment of diagnostic accuracy malaria rapid diagnostic tests and utilisation of malaria parasite result in malaria case management. We would very much appreciate your participation in this study.

A. Purpose of the study

In order to diagnose malaria precisely, we need to do laboratory tests. These tests need to be done in a laboratory. Rapid tests to diagnose malaria within 30 minutes are now available but we do not know if they are accurate or reliable.

The main purpose of this study is to evaluate a rapid test for the diagnosis of malaria. We would like to compare the result of these rapid tests with the results of a laboratory-based microscopy to see if they are as accurate as laboratory tests.

B. Procedures to be followed

If you agree to participate in the study, you will be assigned a study number. We will ask you some questions about your child and will prick her/his finger. We will take about 6 drops of blood from your child. Your name or that of your child will not appear on any specimens or study forms.

C. Voluntary participation

A decision not to participate or to withdraw from participation will not affect the care you will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from study at any time (verbally).

D. Discomfort and risks

Your child may feel a small amount of discomfort or have a small amount of bruising on your finger where the blood was taken. Drawing blood from the arm is usually very safe and only causes discomfort for a short time. It's a routine procedure done in most health facilities and should not cause any harm especially when done by a qualified medical personnel, which is the case in this study.

E. Benefits

You would be able to know whether your child has malaria or not within minutes with the use of the rapid test. However, there is a need to confirm this result by microscopy. When the study results are known and the rapid tests are acceptable in terms of accuracy, everyone who comes to the clinic may benefit from having this test available to diagnose malaria and receive the right treatment the same day.

F. Compensation

There will be no monetary compensation for this study.

G. Confidentiality statement

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

H. Questions and freedom to withdraw from the study

You may withdraw from the study at any time without affecting your present or future medical care at the clinic. You may contact any of the research assistants/health workers if you have questions about the research or call 07035590329.

I. Results publication

Data from the study will be analysed. The results and the explanation of its implications will be given to the health facility and other stakeholders. The information you provide will help the government to plan health services. The survey usually takes between 10 minutes to complete. Whatever information you provide will be kept strictly confidential and will not be shown to other persons.

Finally, participation in this survey is voluntary and you can choose not to answer any individual question or all of the questions. However, we hope that you will participate in this survey since your views are important.

At this time, do you want to ask me anything about the survey?

May I begin the interview now?

Signature/thumb print of patient care giver_____Date: _____

Respondent agrees to be interviewed	➔ Proceed with interview	Respondent does not agree to be interviewed	➔ Go to the next patient

Appendix 2: Sample Questionnaire

Assessment of diagnostic accuracy of malaria rapid diagnostic test and utilisation of laboratory services for Malaria diagnosis at Makarfi general hospital, Kaduna state

Questionnaire No:

Unique Identification No: MKR001 Date: .../…/……..

Name of Health facility:

Section A. Socio-Demographic factors

1. Age of child (in months):
2. Sex of child: ☐ Male ☐ Female
3. Occupation of child's caregiver:
    a. Farmer
    b. Trader
    c. Artisan
    d. Civil servant
    e. Others specify:
4. Religion of child's caregiver:
    a. Islam
    b. Christianity
    c. others
5. Educational status of child's parent:
    a. None
    b. Informal (quranic school) or adult education
    c. Primary

d. Secondary

e. Tertiary

6. Marital status of child's caregiver:

   a. Single

   b. Married

   c. Widowed

   d. Divorced

7. Address of child's caregiver:

8. Distance of residence of child's caregiver from the hospital

   a. <5km

   b. 5-10km

   c. >10km

Section B. Clinical symptoms and signs (tick as appropriate)

9. Fever (axillary Temp: >37.5C ☐
10. History of fever in last 24 hrs ☐
11. Headache ☐
12. Cough ☐
13. Catarrh ☐
14. Chills and or rigors ☐
15. Joint pain and or generalised body pains/ache /weakness ☐
16. Vomiting ☐
17. Diarrhoea ☐
18. Nausea ☐

19. Abdominal pain ☐

20. Bitter taste ☐

21. Others ☐, please specify…….

22. Duration of symptoms: days_____ weeks_____Months_____

History of treatment

23. Have you given your child any form of treatment for fever before presenting at this hospital? Yes/No

24. If yes, is it

    a. Oral drugs

    b. Injection

    c. Herbal preparation

    d. I don't know/cant remember

25. If drug, please state…….

26. How long ago did you give the child the medication? _____ (days)_____(weeks)

Section C. Awareness of laboratory diagnostic services for malaria

27. Have you any heard of any blood test for detection of malaria? Yes/No

28. If yes, mention the test that you know

    a. Microscopy

    b. Rapid test (a test done with a prick done and you get result in 5-10minutes)

Section D. Factors determining utilisation of laboratory diagnostic services

29. How many episodes of fever have this child had or any of your children had in the last 1year?...../Don't know

30. Malaria block / Months of the year (Tick month and state number of episodes)

Months	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
No of Episodes												

31. Did you seek medical care? Yes/No

32. If yes, from where?

    a. Patent Medicine store

    b. Primary health care centre

    c. General hospital

    d. Tertiary health facility

    e. Private health facility

    f. Herbalist

    d. Others, specify

33. When you brought your child with fever to the heath facility, have you ever been asked by a health worker to do a laboratory test for malaria? Yes/No/Don't know

If yes go to 35 or else go to 39

34. If yes, did you accept to do the test? Yes/No/don't know

35. How much did you pay for the test?

    a. Nothing

    b. N100-200

    c. N200-500

    d. N500-1000

    e. >N1000

36. How much do you earn on average in a month?

    a. <N1000

    b. N1000-5,000

    c. >N5000

37. If you did not accept to do the test, what was the reason?

a. It is costly

b. It is not important

c. It will make no difference

d. It takes long for the result to come out

e. It involves pricking and thus painful

38. When last did you do a laboratory test for malaria for your child? ...../never/don't know/can't remember

39. How long did it take between doing test and the release of result?

    a. result was never released

    b. Same day

    c. 2-3 days

    d. ≥4 days

40. What was the result?

    a. Positive

    b. Negative

    c. Don't know

41. If test result was positive, which antimalarial(s) was/were prescribed for your child?

a. SP-3tabs (fansidar, Maloxine, Vitadar…)

b. Chloroquine (the one you take 4 in the morning daily for 2days and 2 tabs the third day)

c. Quinine

d. Camoquine

e. ACT (Lonart, Amatem, Coartem,etc)

f. Don't know

g. Others specify…..

42. Did your child get well after completing the treatment? Yes/No

If yes, skip 43-48

43. If No, did the same symptoms persist? Yes/No

If No, skip 44-48

44. If Yes, did you have to return to hospital? Yes/No

45. After how many days did you have to return to hospital complaining of same symptoms?

a. Same day

b. Next day

c. 2days after

d. 3days after

e. 4 days or more after

f. Don't know/can't remember

46. Were you prescribed another type of antimalarial? Yes/No

47. If yes, please state drug ……

48. Were you prescribed another drug apart from antimalarial? Yes/No

49. If yes, state please…….

Appendix 3: Case report form for rapid diagnostic test and microscopy

Laboratory results

Malaria RDT Results (Finger-Prick)

1. Site ID#_____

2. Specimen Number**:**_____

3. Specimen Date: [____/____/_____](dd/mm/yyyy)

4. SD Bioline RDT

a. Positive

b. Negative

c. Invalid

5. Malaria Microscopy Results

Microscopy (thin/thick film) 1. Positive 2. Negative 3. Not done (Reasons….)

If microcopy is positive, state species of *Plasmodium*

a. *P. falciparum*

b. *P. vivax*

c. *P. ovale*

d. *P. malariae*

e. Not done (Reasons….)

Parasite count:

6. Laboratory scientist/technician's

Signature _____Date_____

Appendix 4: Patient rapid diagnostic test result slip

Laboratory results

Malaria RDT Results (Finger-Prick)

Patient's name: Age: Sex:

1. Site ID#_____

2. Specimen Number**:**_____

3. Specimen Date: [____/____/_____](dd/mm/yyyy)

4. SD Bioline RDT

a. Positive

b. Negative

c. Invalid

Appendix 5: Checklist for assessing the capacity of laboratory staff on malaria microscopy and status of the laboratory

1. No of laboratory(lab) staff
2. Years of experience /No of staff:
    a. <2years/…
    b. 2-5yrs/…
    c. >5yrs/…
3. Age range of lab personnel (yrs)
    a) <20
    b) 20-30
    c) 30-45
    d) >45
4. Qualification/Number (No) of lab staff
    a. AIMLS(T)/…
    b. FMILS(T) /…
    c. B.Sc/…
    d. BMLS/…
    e. HND/…
    f. (Others (specify) /…
5. How many lab staff was trained on malaria microscopy?
6. Which kind of training did they receive?
7. Level of training on malaria microscopy/No of staff
    a. WHO Certification stage 1/…

b. WHO Certification stage 2/…

c. WHO Certification stage 3/…

8. No of malaria doctor's/health-provider's requests/day……..

9. Average number of slides for malaria parasite

   a. <5 slides/day

   b. 6-10 slides/day

   c. 11-20 slides/day

   d. >20 slides/day

10. Are Standard Operating Procedures (SOPs) available?

11. If yes, state the date of development…….

12. When was it last reviewed?

13. Are SOPs in use?

14. Have you experience stock-outs of reagents and supply in the past 3 months?

15. What is the main source of power in the laboratory?

    a. Sunlight

    b. Electricity

    c. Generator-powered

16. What is the main source of power for the microscope

    a. Sunlight

    b. Electricity

    c. Generator-powered

17. Availability of equipments: list and tick

18. State of equipment, materials and other supplies

Score each of the item by awarding "0" (not available or not functioning) to "4" (in excellent working condition).

S/N	Equipment	Number available	State the No that are functioning	Remarks
1.	Binocular Microscope (Reichart Jung, Olympus, Nikkon) with illumination			
2.	Spare Microscope bulbs			
3.	Lens paper			
4.	Haematocrit Centrifuge (Hawksley) with reader			
5.	Stop Clock/laboratory timer			
6.	Thermometers			
7.	Double Tally Counter			
8.	Microscope slides, Frosted edge, pack of 100			
9.	Slides tray cardboard			
10.	Slide box for vertical storage (capacity 100)			
11.	Slide box for horizontal storage (capacity 100)			
12.	Hair dryer (for humid areas and seasons)			
13.	Slide (drying) racks			

14.	Indelible slide markers			
15.	Immersion oil (Anisole),bottle of 50ml			
16.	Staining jar, coplin or horizontal			
17.	Bottle screw-cap, plastic,500ml			
18.	Bottle or jerry can, screw cap, plastic,5L			
19.	Measuring cylinder, plastic 500ml			
20.	Measuring cylinder, plastic 10ml			
21.	Pipette,transfer,disposable,5ml pack of 100			
22.	Pipette,transfer,disposable,1ml pack of 100			
23.	Dropping-bottle, plastic or glass,50ml			
24.	Glass rod,50cm,for quick staining			
25.	Giemsa Stain Stock solution, bottle of 500ml			
26.	Buffer Tablets,Ph 7.2			
27.	Haemolancets, pack of 100			
28.	Heparinised capillary tubes, pack of 100			
29.	Filter paper (Whatman) pack of 100			
30.	Lens tissue, pack of 100			
31.	Methylated spirit			
32.	Swabs, alcohol(70%),pack of 100			

33.	Cotton wool,pack of 500g			
34.	Tissue paper,rolls			
35.	Rubber Gloves, disposable, medium size			
36.	Rubber Gloves, disposable, large size			
37.	Glass-writing pen, permanent, xylene proof			
38.	Adhesive writing stickers			
39.	Plasticine (Hawskley)			
40.	Xylene, bottle of 500ml			
41.	Methanol, bottle of 500ml			
42.	Syringe,plastic,10ml			
43.	Distilled water			
44.	Logbooks			
45.	Notebooks			
46.	Patient forms			
47.	Patient cards			
48.	Laboratory form (microscopy and haematology)			
49.	Adhesive tape,roll,30cm			
50.	Ball-point pen, black/blue			
51.	Ball-point pen, red			

Appendix 6: Focus Group Discussion guide for assessment of factors affecting utilisation of laboratory services for malaria at Makarfi community

Date: …….

Name of recorder (note-taker):……..

Name of moderator:……

Name of interpreter:……

Availability of tape recorder: Yes/No……

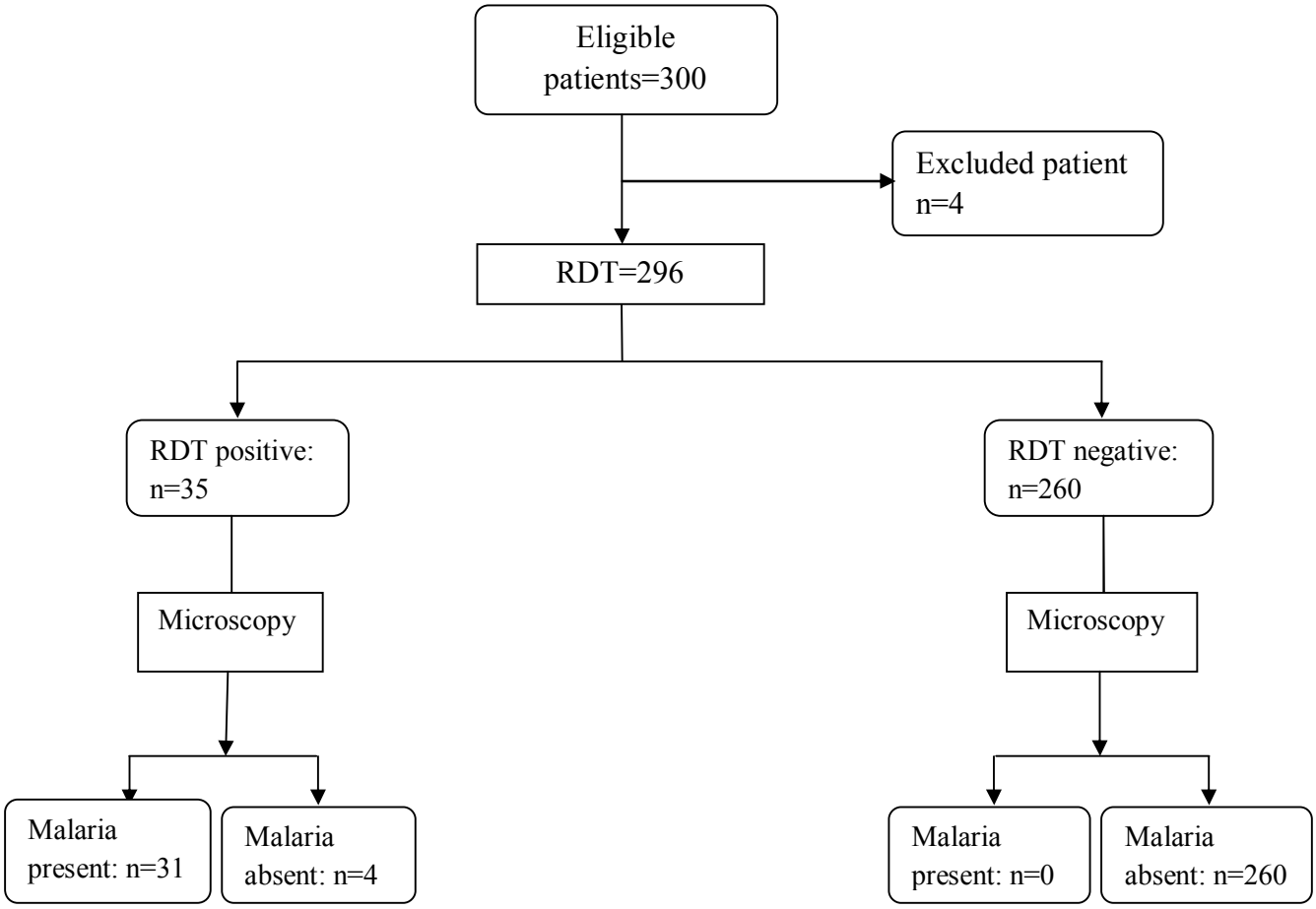
Village/community:.…… Number of participants: ……

Type of Group: female /male:…….. Age range: ……

Female: mothers of children under 5: ……

1. Male: fathers of children under 5: …… Please tell us about the general health problems in this community and the most common diseases among : Children/ Adults / Pregnant women
2. What is malaria?
3. Does malaria affect people in this community and to what extent?
4. When people in this community have malaria how did they treat it?
5. Before being given drugs do they check your blood for the disease?
6. What makes people to use laboratory services?
7. What makes people not to use laboratory services?

Appendix 7: Flow chart for diagnostic accuracy part of the study



Appendix 8: Definition of variables

Case definitions: [111]

Uncomplicated Malaria: Any person with fever or fever with headache, back pain, chills, sweats, myalgia, nausea, and vomiting diagnosed clinically as malaria.

Confirmed uncomplicated malaria: Any person with fever or fever with headache, back pain, chills, sweats, myalgia, nausea and vomiting and with laboratory confirmation of diagnosis by malaria blood film or other diagnostic test for malaria parasites.

Severe malaria**:** Any person hospitalised with a primary diagnosis of malaria and confirmed by a positive blood smear or other diagnostic test for malaria. Any febrile child unable to eat or drink, dehydrated and anaemic.

Malaria with severe anaemia**:** Any child 2 months up to 5 years with malaria and, if an outpatient, with severe palmar pallor, or if an inpatient, with a laboratory test confirming severe anaemia.

Appendix 9: Laboratory methods

1. Rapid diagnostic test: RDTs are lateral flow Immunochromatographic antigen-detection tests (ICT), which rely on the capture of dye-labeled antibodies to produce a visible band on a strip of nitro-cellulose. These tests are based on the detection of antigens derived from malaria parasites in lysed blood, using Immunochromatographic methods. Most frequently they employ a dipstick or test strip bearing monoclonal that target parasite antigens. In the case of malaria RDTs, the dye-labeled antibody first binds to a parasite antigen and the resultant complex is captured on the strip by a band of bound antibody, forming a visible line (test line). A control line gives information on the integrity of the antibody-dye conjugate [13, 23]. *Plasmodium falciparum* specific RDTs were used. These RDTs interact with Histidine-rich protein II (HRP-II), an antigen produced by plasmodium falciparum. HRP-II is a water-soluble protein produced by trophozoites and young gametocytes of *P. falciparum*. The patient's ring finger was swabbed with an alcohol wipe and pricked using a lancet. The loop was used to collect a drop of blood. This was then dropped into a square hole marked on the RDT cassette. Six drops of buffer were added into the round hole and read the results after 15 minutes. **POSITIVE result is shown when** One red line in window **"C" (Control) AND** one red line in window **"T" (Test)** means the patient **DOES** have *falciparum* malaria. The test is **POSITIVE** even if the red line in window **"T"** is faint. **For a NEGATIVE result: One red line** in window **"C"** and **NO LINE** in window **"T"** means the patient **DOES NOT** have *falciparum* malaria. **INVALID RESULT: NO LINE** in window **"C"** means the test is damaged. A line in window **"T"** and **NO LINE** in window. **"C"** also means the test is damaged. Results are **INVALID**. The results of the RDT were read within 15minutes.

2. Microscopy: Thin and thick blood films stained with Giemsa were examined for each eligible patient and for the identification and differentiation of Plasmodium falciparum and other species.

Thick Film: contains many layers of red and white blood cells. It was used for the initial search for parasites. The thin film was used to confirm malaria parasite species.

The results of the blood film were processed daily otherwise every other day depending on workload.

Preparation of a thin and thick film:

Materials needed were purchased and prepared (and quality assured with the help of experienced malaria microscopist) by the investigator. These were:

1. Latex gloves
2. Frosted end slides
3. Sterile lancets
4. 70% ethanol
5. Absorbent cotton wool
6. Sharps container
7. Slide box
8. Lint-free cloths
9. Record forms and register
10. Ball-point ink-pen
11. Lead pencil
12. Sharpener

After obtaining informed consent from the patient and wearing protective latex gloves, the patients' left hand with palm facing upwards was held. Ball of the third (ring) finger was cleaned with cotton wool dabbed in alcohol and wiped dry with dry cloth. This was then punctured with a sterile lancet. A gentle pressure was applied and the first drop of blood was wiped away with a dry cloth. Another gentle pressure was applied to the finger and a single drop of blood was collected on the middle of the slide. Two to three larger drops were then collected on the slide 1cm away from the drop intended for the thin film. The remaining blood was wiped off with cotton wool.

Thin film: another clean slide (spreader) held at an angle of 45° touching the drop of blood and the surface of the other slide and dragged allowing the blood to run right along the edge.

Thick film: the slide was held at the side and the spreader was used to join the 3drops of blood together to make an even, thick film. The thick film was dried level away from sunlight, extreme heat, dust and flies.

Giemsa stain is an alcohol-based Romanowsky stain. It is a mixture of eosin which stains the malaria parasite chromatin and stippling shades of red or pink and methylene blue, which stains parasite cytoplasm blue. The Giemsa-stained slide was examined under light microscope.[109, 110]

Appendix 10: Determination of parasite density

Parasite density in the thick blood smear was assessed by counting the number of asexual parasites (and the number of white blood cells in a limited number of microscopic fields) against ≥200 leucocytes in a thick film, and converting the value to parasites/µl using the standard of 8,000 leucocytes/µl. Hand tally-counters were used and a field was always counted to the end. If 500+ parasites have been counted without having reached 200 leucocytes, the count was stopped after completing the reading of the last field, and the parasitaemia was calculated according to the formula below.

$$\text{Parasite density (number of parasites/µl)} = \frac{\text{Parasite count x 8,000}}{\text{No of WBC counted}}$$

When the number of asexual parasites has dropped below 10 parasites per 200 leucocytes, counting was done against 500+ leucocytes (i.e. to the completion of the field in which the 500th leucocyte has been counted). A blood slide was deemed negative when the examination of 100 thick film fields did not show the presence of asexual forms of *P. falciparum*. In addition, 100 fields of the thick film were examined for the exclusion of mixed infections**.** The presence of *P. falciparum* gametocytes was not part of the evaluation of the test. [26, 31, 89]

Appendix 11: Ethical certificates

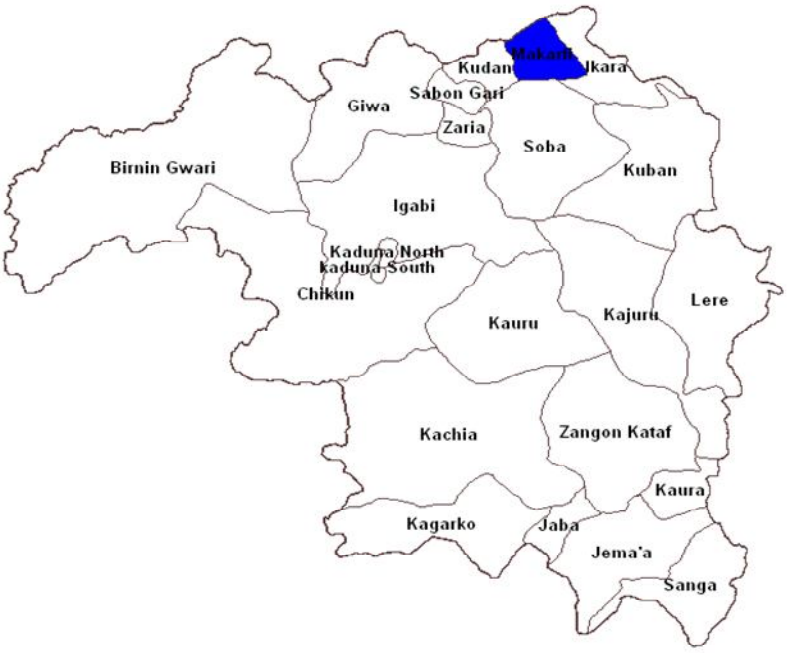


Figure 6: Map showing Kaduna state, the LGAs and Makarfi