

SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA

**DIAGNOSTIC ACCURACY OF MALARIA RAPID TESTS IN ROUTINE FIELD
SETTINGS IN KASSENA-NANKANA DISTRICT OF NORTHERN GHANA:
IMPLICATIONS FOR THE TEST AND TREAT POLICY**

BY

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DECLARATION

I, Alfred Bornwell KAYIRA, declare that except for other people's investigations which have been duly acknowledged, this study dissertation is the result of my own work undertaken under supervision, and that it has neither in whole nor in part been presented for another degree in this university or elsewhere.

Signed: Date

Alfred Bornwell KAYIRA

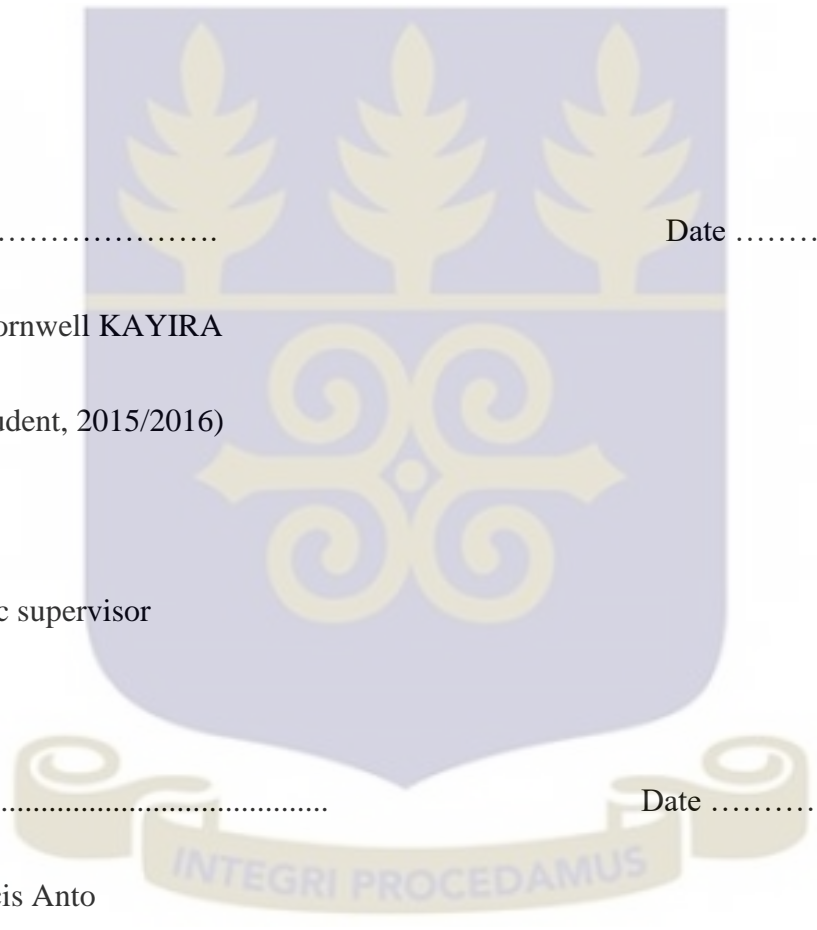
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DEDICATION

This work is dedicated to my spouse, Lusungu Beatrice Kyusa, mam and dad and to my brothers and sisters. Thanks for being there.



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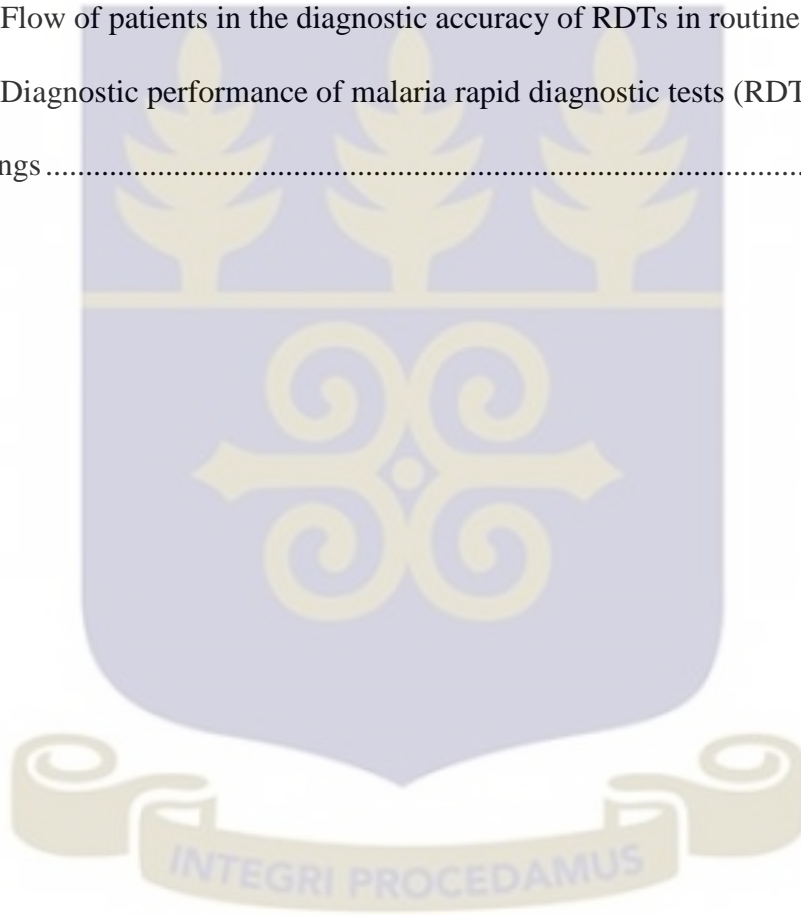
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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
CI	Confidence interval
EDTA	Ethylenediaminetetraacetic acid
HRP-2	Histidine-rich protein 2
PfHRP-2	Plasmodium falciparum histidine-rich protein 2
pLDH	Plasmodium Lactate dehydrogenase
NMCP	National Malaria Control Programme
NHDSS	Navrongo Health and Demographic Surveillance System
RDT	Rapid Diagnostic Test for malaria
WHO	World Health Organization



ABSTRACT

Rapid diagnostic tests for malaria (RDTs) carry the hope that malaria treatment can be prompt, accurate and cost-effective. However, malaria rapid tests can achieve this goal only if they give accurate results comparable to those recommended by World Health Organization, and if health workers apply treatment according to test results. The study evaluated the diagnostic accuracy of malaria rapid tests in routine field settings and the implications for the test-based management of malaria. Out patients presenting for a malaria test requested by their doctor were serially enrolled into the study. Their folder/OPD numbers were noted. A finger prick blood sample for thick and thin film slides was collected in a micro-EDTA tube soon after a blood sample for a malaria rapid test had been collected by the health staff performing the test from the same finger prick site. Malaria rapid test results, as read and interpreted by the person who did the test, were recorded and later compared to expert microscopy which was taken as the gold standard. A structured case record form was used to collect primary data on factors with the potential to affect the diagnostic performance of RDTs in those settings. Patients' folders were later retrieved from the dispensary or records department. Diagnosis, prescription and medicines dispensed were extracted from the folders. Prevalence of parasitaemia was 5%, with *P. falciparum* as the only species transmitting and infecting people at the beginning of the high transmission season. Sensitivity, specificity, positive predictive value, negative predictive value, false positive rate and false negative rate were 69.6%, 92.4%, 32.7%, 98.3%, 7.6%, 30.4%, respectively. Parasitaemia of $\leq 100/\mu\text{L}$ was the single lone predictor of low sensitivity. Body temperature of $\geq 37.5^\circ\text{C}$ was found to have a rather bizarre association with low specificity which has no plausible biological explanation. Prescriber

adherence to both positive and negative RDT results was 100%. RDTs have substantially improve the targeting of ACTs to malaria patients. However, their diagnostic performance is insufficient to be a standalone method upon which to wholly base clinical decision. They must be complemented with other better performing diagnostic technologies and other setting-specific malaria management guidelines to sustainably support the Test and Treat strategy.



CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria, though a preventable and curable disease, remains a major global public health problem, particularly in Sub-Saharan Africa where 90% of global cases occur with highest morbidity and mortality being in pregnant women and children under the age of five (WHO,2014). In Ghana malaria is the number one cause of morbidity accounting for about 32% of all outpatient consultations and 48.8% of under-five admissions in the country (NMCP annual report, 2009). Prompt and accurate diagnosis of malaria is key to effective case management, preventing morbidity and mortality (WHO, 2010).

Malaria rapid diagnostic tests (RDTs) have the potential to improve prompt and accurate diagnosis of malaria, particularly at peripheral level of the health system with no access to parasite-based laboratory test. Improving diagnosis and treatment for malaria will in turn improve treatment outcomes, rationalize health care costs by reducing drug consumption, minimize drug pressure that can lead to resistance, and assist in monitoring disease trends (Dondorp et al., 2010; Naidoo et al., 2010).

In 2010 World Health Organization (WHO) recommended that every antimalarial dispensed should be preceded by a parasite-based confirmation test (WHO, 2010). By 2012, 41 out of 44 endemic countries in the WHO Afro Region, including Ghana had adopted the policy of providing malaria diagnostic testing for all age groups before treatment (WHO, 2013), but with little or no quality assurance protocols (McMorrow et

al., 2008). Today, mainly because of their relative inexpensiveness and ease of use with minimal training, RDTs have become the staple test method for malaria diagnosis, particularly at lower levels of the health system which before then had no access to parasite-based malaria diagnostic test (Mayxay et al., 2004).

However, full potential of RDTs as regards to cost-effectiveness, case management and disease surveillance can be realized only if they give accurate results and prescribers apply treatment according to test results (Abeku et al., 2008). Like any other test, the accuracy (sensitivity and specificity) of RDTs however, depends on product factors and end user factors (Abeku et al., 2008). WHO undertakes evaluation exercises of most of the commercially available RDTs and approves only those with the highest sensitivities and specificities ($\geq 95\%$) for clinical use (WHO, 2014). However assessment of sensitivity and specificity of RDTs in field settings where routine testing takes place has unsatisfactorily been done, hence underscoring the impact of setting-specific factors on test performance. By ignoring setting-specific factors we could be dangerously working with the assumption that the performance of RDTs in routine field settings have similar performance to that observed by WHO in their test validation and verification exercises.

1.2 Problem statement

The introduction of rapid diagnostic tests (RDTs) for malaria has revolutionized the way malaria is diagnosed and treated in malaria-endemic sub-Saharan Africa including lower levels of the health system where initially diagnosis was empirical based solely on clinical symptomatology. To reduce over diagnosis and treatment of malaria World Health Organization (WHO) in 2010 recommended that all suspected malaria cases should be

confirmed with a parasite-based diagnostic assay as opposed to diagnosis based on clinical symptoms before treatment with antimalarial drugs in order to reduce cost on the relatively expensive current Artemisinin-based Combination Therapies (ACTs) used to treat malaria, and to prevent or delay the emergency of resistance to these drugs (WHO, 2010). Currently 78 (91%) of the 86 countries where *Plasmodium falciparum* is endemic have adopted policies to confirm malaria with a laboratory test prior to treatment in all age groups and have deployed RDTs in all health facilities including those at the lowest level in rural areas (WHO Malaria Report, 2010).

The expectation is that in routine field settings malaria rapid diagnostic tests will continue to perform to the same levels of sensitivity and specificity as observed in test panel validation protocols. However, this may not be true especially now that sub-Saharan Africa is experiencing a significant decline in malaria burden leading to frequent occurrence of light infections with parasite densities below the detection threshold of ≥ 200 parasites per microliter by RDTs (Bharti et al., 2008; WHO Malaria Report, 2010), but which are still deadly to children under the age of five and pregnant women. Sensitivity of RDTs depend to some varying degrees on the *Plasmodium* species endemic to a particular region, prevalence of genetic polymorphisms and gene deletion, and levels of parasitaemia expressed by different species in infected individuals (Fransisca, 2015). During high transmission seasons RDT specificity is also affected by the histidine rich-protein-type 2 (HRP-2) antigens which may persist in previously infected and treated individuals' circulation for more than 30 days leading to high rates of false positive (Iqbal et al., 2004; Kyabayinze et al., 2008; Abeku et al., 2008). Accuracy of RDTs is further influenced by the expertise of the person performing and reading the test (Chinkhumba et al., 2010).

Being a serological test RDTs are more susceptible to extreme environmental conditions such as high temperatures and humidity (Wongsrichanalai et al., 2007; Chiodini et al., 2007a). Hence, stringent quality assurance activities to monitor the viability of RDTs over time need to be in place if we are to have sustainable quality RDT results, but this is rarely the case in lower level health facilities.

Unfortunately it is the lower level health facilities which are usually vulnerable to factors which compromise the accuracy of a malaria rapid diagnostic test, including lack of confirmatory test, less skilled health workers, lack of control over environmental conditions under which RDTs are kept, absence of quality control procedures. Further, it is the same facilities which have demonstrated good adherence to positive RDT results when prescribing ACTs (Manyando et al., 2014) .

Assuming that RDTs in peripheral facilities have sensitivities and specificities comparable to those obtained in clinical trials or those claimed by manufacturers could be wrong because the diagnostic accuracy of RDTs is also influenced by factors specific to a particular setting. Inappropriately identifying patients as having or not having malaria underscores the overall goal of introducing malaria rapid tests which is to improve rapid case detection and treatment, cost-effectiveness in malaria case management, minimize drug pressure and delay resistance, and to improve malaria case surveillance. Further, unreliable results undermines user confidence and trust in the test method and make them more unlikely to comply with test results when prescribing antimalarials.

1.3 Objectives

1.3.1 General objective

To evaluate the diagnostic accuracy of malaria rapid tests in routine field settings in Kassena-Nankana District of Northern Ghana, and the implications for the test-based management of malaria

1.3.2 Specific objectives

1. To determine *Plasmodium* species prevalent in Kassena Nankana District of Northern Ghana at the beginning of the high transmission season.
2. To determine the sensitivity and specificity of malaria rapid diagnostic tests in routine field settings
3. To explore factors that affect sensitivity and specificity of malaria rapid tests in routine field settings.
4. To assess prescriber adherence to rapid diagnostic test results when prescribing antimalarial drugs in those settings

1.4 Research questions

1. What are the *Plasmodium* species in transmission in Kassena Nankana District of Northern Ghana at the beginning of the transmission season?
2. What is the sensitivity and specificity of malaria rapid diagnostic test kits in routine field settings?

3. What are the factors affecting the sensitivity and specificity of malaria rapid tests in routine field settings?
4. Are prescribers adhering to rapid diagnostic test results when prescribing antimalarial drugs in these settings?

1.5 Rationale for the study

Prompt and reliable diagnosis is critical for malaria case management, and prevents overdiagnosis and underdiagnosis of malaria. Overdiagnosis due to poor specificity of the test leads to overprescription of the relatively expensive Artemisinin-based Combination Therapies (ACTs) which is not only costly but also accelerates the emergency of resistance due to drug pressure. On the other hand underdiagnosis (missed or delayed) due to poor sensitivity of the test delays the much needed treatment and has poor prognosis. Infected and untreated individuals become the reservoir of infection which supports the transmission cycle of malaria. Both overdiagnosis and underdiagnosis have huge implications for disease surveillance.

Lower level health facilities are the most affected by inaccurate RDT results because of the lack alternative or confirmatory test method, less skilled personnel, extreme storage conditions of test kits, lack of quality control procedures. Inaccuracies and inconsistencies in test results undermines both provider and patient confidence in the test method which in turn leads to poor adherence to test results when prescribing antimalarials.

Investigating and demonstrating the diagnostic accuracy of RDTs will help users (prescribers and patients) accept and build trust in test results, and apply treatment

according to test results. The level of accuracy observed will also prompt health managers, policy makers and stakeholders to reevaluate the presumed cost-effectiveness of RDTs. The study also intends to explore support structures which can help RDTs realize their full potential.



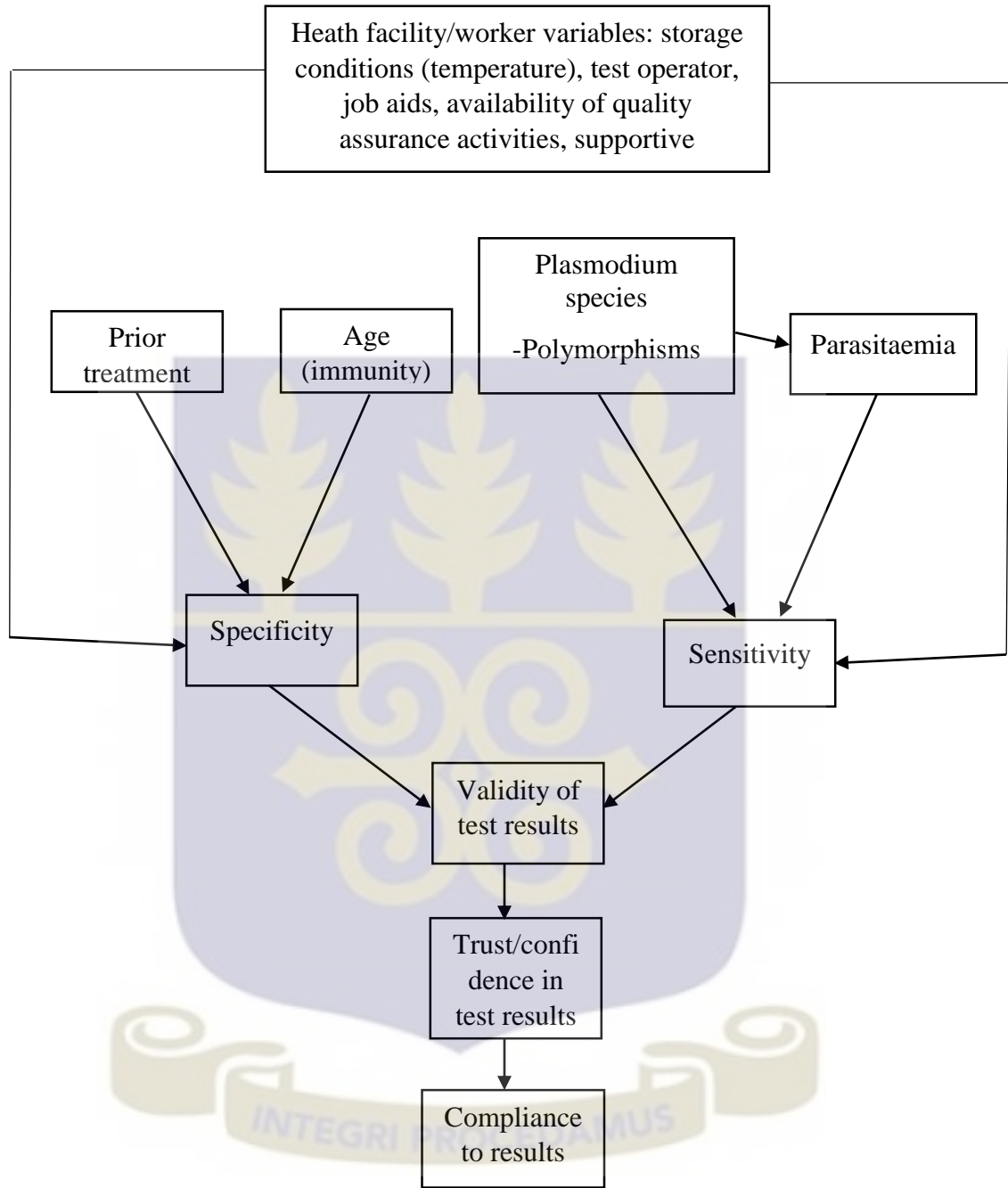


Figure 1. Conceptual framework

Narrative

Factors that affect diagnostic accuracy in real world settings can be grouped into three categories: health facility/worker factors, patient factors and parasite factors. Facility factors include storage conditions of test kits (temperature) which affect stability of the test devices over time. Health worker factors refer to whether the test operator is trained and competent, and that he is able to correctly perform the test, read and interpret test results according to manufacturer's directions. Quality assurance system monitors the performance of test devices over time and identifies performance deviations in time. Supportive supervision identifies deficiencies in the test operator and institutes timely training/retraining.

Patient factors include age and prior treatment with antimalarials. Infants have under-developed immunity, take longer (up to 30 days) to clear antigens from previous infections and will continue to test positive for a relatively longer period even if the infection was successfully treated. Similarly, prior use of antimalarials: RDT results remain positive for at least two weeks despite successful treatment.

Parasite characteristics affect sensitivity of RDTs in the sense that not all RDTs detect all plasmodium species. Even those kits that detect a specific specie (*P. falciparum*) may at some point in time (or in some regions) not be able to sufficiently detect it due to polymorphisms and gene deletion. Detection rate of RDTs also begin to stagger when parasitaemia falls below 200 per microlitre of blood.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global and region burden of malaria

Malaria remains one of the most common diagnoses in sub-Saharan Africa, and fever the commonest outpatient presentation. Although malaria is a preventable and curable disease, there were an estimated 198 million cases of malaria globally in 2013, and the disease led to 584 000 deaths. Up to 90% of the deaths occurred in sub-Saharan Africa, and most of them (78%) in children under 5 years of age (WHO, 2014). According to WHO, one child dies from malaria every minute in Africa. Globally, an estimated 3.3 billion people are at risk of being infected with malaria and developing disease out of which 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year) (WHO, 2014).

2.2 Global and regional efforts to combat malaria

Global efforts to control malaria are also scaling up. International and domestic funding for malaria control and elimination totaled US\$ 2.7 billion in 2013 representing a threefold increase from 2005 (WHO, 2014). WHO African Region accounted for 72% of the total global funding for malaria. Between 2005 and 2013, international disbursements for malaria for this region increased at an annual rate of 22%, and during the same period, the average annual rate of increase for domestic funding in the region was 4% (WHO, 2014).

The global war against malaria is being fought from three fronts: vector control, chemoprevention and case detection and management. Integrated vector management

methods include the use of insecticide treated mosquito bed nets, indoor residual spraying and larviciding. Sub-Saharan Africa has for the past ten years seen a substantial increase in coverage with vector control interventions. By 2013, almost half of the population at risk had access to an insecticide-treated mosquito net (ITN) in their household, and the proportion of the population protected by at least one vector control method reached 48% (WHO, 2014).

Intermittent Seasonal Chemoprevention is being advocated for pregnant women and children below the age of five years. Chemopreventive therapy has been adopted by 35 countries of the WHO region. The proportion of women who receive intermittent preventive treatment in pregnancy (IPTp) for malaria in those countries has been increasing over time and reached 57% in 2013 (WHO, 2014).

Malaria case diagnosis and treatment is the most crucial and delicate battle front in as far as the war against malaria is concerned. This is because health providers have to balance between patient optimal outcomes and rational use of the relatively expensive Artemisinin-based Combination Therapies (ACTs) so as to minimize treatment costs and offset drug pressure which ultimately leads to drug resistance. In 2010 WHO recommended that malaria case diagnosis and treatment should be guided by a parasitological test (WHO, 2010). To date, many malaria endemic countries in sub-Saharan Africa have adopted and introduced RDTs at every level of their health system to guide diagnosis and treatment, and to strengthen surveillance. However, access to diagnosis remains poor in half of

endemic African countries, and over 80% of malaria treatments are applied without diagnostic testing (WHO, 2013).

In April 2012, the World Health Organization's (WHO) Global Malaria Programme embarked on a highly ambitious new initiative called T3: Test. Treat. Track (WHO, 2013). T3 aims to address the widespread problem of poor access to diagnostic testing and antimalarial treatment, and to enhance case-reporting. The initiative had set a target of universal access to diagnostic testing prior to treatment in the public and private health care sector by 2015 (Who, 2013). Achieving this goal entirely hinges on the use of malaria rapid diagnostic tests (RDTs).

The cost-benefit and accurate case identification and reporting provided by RDTs will however, depend on whether they are able to accurately distinguish between patients with malaria and those without, and whether prescribers apply treatment according to test results. Often, the irrational use of tests and drugs is based on perceived shortcomings of the tests. A common concern amongst health staff is that negative tests do not always exclude malaria (WHO, 2014).

2.3 Technology of rapid diagnostic tests for malaria

Malaria RDTs are lateral flow devices that use antibodies to capture and detect parasite proteins by immunochromatography. RDTs for malaria are based on the detection of either histidine-rich protein 2 (HRP-2), produced only by *Plasmodium falciparum*, parasite specific lactate dehydrogenase (pLDH) produced by all four species or plasmodium aldolase from the parasite glycolytic pathway, also found in all species. One hundred and

ten of the 127 RDTs brands that are capable of diagnosing *Plasmodium falciparum*, target HRP-2 (WHO, 2014). Tests based on detection of pLDH or aldolase allow parasite speciation

2.4 Accuracy of RDTs

Currently, over 200 brands of RDTs are commercially available for detecting different *Plasmodium species*. WHO and other organizations (Centers for Disease Control and Prevention (CDC), Foundation for Innovative New Diagnostics (FIND), Special Programme for Research and Training in Tropical Diseases (TDR), undertake yearly product-quality testing. Over the past five years 128 unique RDT products have been tested. Only those products which have demonstrated sensitivities and specificities of at least 95% are approved for clinical use (WHO, 2014). In real field settings, however, RDTs have demonstrated substantial variability in their sensitivities and specificities (Abeku et al., 2008; Azikiwe et al., 2012; Baiden, 2012; Chinkhumba et al., 2010; Mills et al., 2010; Tarimo, 2015). Setting-specific factors may be responsible for the observed variability.

In two comparative studies conducted by Ishengoma (2011) in Tanzania, one longitudinal and another cross-sectional, sensitivities and specificities of RDTs varied widely. In a longitude study sensitivity and specificity when compared to microscopy as the gold standard were 88.6% and 88.2%, respectively. In the cross-sectional study, the sensitivity was significantly lower (63.4%; $p < 0.001$), while the specificity was significantly higher (94.3%; $p < 0.001$) when compared to the longitudinal study. These studies identified parasite density of <200 asexual forms per microlitre and presence or absence of fever

(axillary temperature of $\geq 37.5^{\circ}\text{C}$) on consultation, and seasonality as determinants of sensitivity and specificity respectively.

Accuracy of RDTs has been shown to be influenced by endemicity or level of transmission and age of patient besides parasite density and presence or absence of fever during consultation (Abeku et al., 2008). This study evaluated the performance of RDTs at two sites: a hypoendemic site and a mesoendemic site, with microscopy as the reference test method. The observed sensitivity, specificity, positive predictive value and negative predictive value of RDTs in a hypoendemic site were 90.0%, 99.9%, 90.0% and 99.9%, respectively. Corresponding measures at a mesoendemic site were 91.0%, 65.0%, 71.6% and 88.1%. It can be observed that although sensitivities at the two sites were somewhat comparable levels of specificity varied considerably between the sites. In this study variation was also observed according to month of test, age of patient, and presence or absence of fever during consultation. Specificity was relatively high in older age groups and increased towards the end of the transmission season.

In preparation to scale-up RDTs in health facilities in Malawi, an evaluation of RDTs to help guide national-level decision-making was conducted (Chinkhumba et al., 2010). Four brands of RDTs, Bioline SD, First response malaria, Paracheck and ICT diagnostics were evaluated against microscopy as the gold standard. All of the brands tested demonstrated relatively high sensitivity for detecting parasitaemia of ≥ 200 asexual forms per microlitre of blood: Bioline SD (97%), First response malaria (92%), Paracheck (91%), ICT diagnostics (90%). Their specificities were however, substantially low and worrisome: Bioline SD (39%), First response malaria (42%), Paracheck (68%), ICT diagnostics (54%).

This study identified two more factors that contributed to poor specificity of RDTs: previous treatment with an antimalarial and cadre of health staff performing the test. Specificity was significantly lower in patients who self-treated with an anti-malarial in the previous two weeks (OR 0.5; p-value < 0.001), and when the RDT was performed by a community health worker versus a laboratory technician (OR 0.4; p-value < 0.001).

Failure to express the antigen by the parasite due to gene deletion or frame shift mutation or alteration in protein has been shown to contribute to variable performance of RDTs. (Bendezu et al., 2010; Koita et al., 2012; Kumar et al., 2013; Lee et al., 2006a; Maltha et al., 2012). These are generally spontaneous mutations which result in deletion of the Histidine-Rich Repeat Region of the hrp2 Gene that codes for histidine rich protein-2 (HRP-2), a target marker (antigen) for most RDTs. The situation is further compounded by genetic polymorphisms in diagnostic antigens (HRP-2) resulting from frame shift mutations (Baker et al., 2005, 2011; Lee et al., 2006a; Mariette, Barnadas, Bouchier, Tichit, & Ménard, 2008). Such altered proteins (antigens) are not detected by monoclonal antibodies in the available RDTs which target an epitope in either HRP-2 or HRP-3 leading to false negativity.

Environmental conditions such as high temperature and humidity are said to affect the stability of RDTs (Chiodini et al., 2007; Murray et al., 2008). High temperatures of more than 35°C have demonstrated to accelerate degradation of RDTs. RDTs targeting the histidine-rich protein of Plasmodium falciparum are particularly vulnerable (Chiodini et al., 2007; Murray et al., 2008). Chiodini (2007) recommends that thermal stability of RDTs

must be an integral component in the selection of the product if it is to be used in the tropics like sub-Saharan Africa.

2.5 Adherence to test results when prescribing antimalarial by health workers

The cost-benefit of RDTs can only be seen when prescribers dispense ACTs according to test results. However, adherence to test results appears to be a big challenge with most health staff. One study in Malawi observed that health workers in settings where both microscopy and RDTs were available adhered to microscopy and RDT positive results and prescribed antimalarials for over 98% of patients who had positive test results (Chinkhumba et al., 2010). Further, health workers prescribed antimalarials for almost all patients with discordant results (microscopy positive and RDT negative). Conversely, in both settings, health staff rarely withheld treatment in instances where RDT results were negative. Up to 58% of patients with a negative RDT results were still treated with an antimalarial in settings where only RDTs were available, but only 7% of patients who were both microscopy-negative and RDT-negative were prescribed an antimalarial in settings with both microscopy and RDTs (Chinkhumba et al., 2010), implying that health workers have more trust in Microscopy as compared to RDTs.

Another study in Zambia explored the role of RDTs in malaria diagnosis and the health workers' adherence to test results. The study found that out of 2, 264 children who had RDT results two in three (68.6%) were treated with an antimalarial despite negative results (Manyando et al., 2014). Still in Zambia, a study by Hamer (2009) found that an antimalarial was dispensed to 96.6% of patients who had a positive RDT result. However, 35% of patients with a negative RDT result were also prescribed an antimalarial despite

the negative result. In Burkina Faso a study was undertaken to determine the impact of the introduction of RDTs on clinical decisions. Between 79.8% and 85.0% of patients with a negative RDT result were nevertheless diagnosed and treated for malaria (Bisoffi et al., 2009).



CHAPTER THREE

METHODS

3.1 Study design

A descriptive cross-sectional study was carried out in three health facilities in the Kassena-Nankana West and East districts. Patients who had been requested by a clinician to take a malaria rapid test were consecutively enrolled into the study. A finger prick blood sample for thick and thin film slides was collected into a micro EDTA tube soon after a blood sample for a malaria rapid test had been collected by the health staff performing the test from the same finger prick site. Malaria rapid diagnostic test results, as read and interpreted by the health worker who did the test, were recorded. Patients' Folder/OPD numbers were noted. These folders were retrieved from the dispensary or Records Department the following day. Information on diagnosis made and medicines prescribed and dispensed was extracted from the folders. The micro EDTA blood samples were couriered to a Central Microscopy Laboratory at War Memorial hospital for film preparation and examination.

Facility and human factors which have the potential to affect the performance of rapid tests were explored. Minimum and maximum temperatures were measured each day for the entire study period. Availability of support systems such as external quality assurance activities and supervision was sought. The test operator was observed performing the test, assessed and scored against manufacturer's test procedures. Cadre, training and years of experience with RDTs of the test operator was noted.

3.2 Study area

3.2.1 Geographic profile

The study was undertaken in Kassena Nankana East and Kassena-Nankana West Districts of Northern Ghana. The two districts were formerly one. It was in 2008 that the district was split into Kassena-Nankana East and Kassena-Nankana West for administrative reasons. In this study, unless otherwise specified, Kassena-Nankana district refers to both of them (before the split).

Kassena Nankana District lies between latitude 10°30' and 11°00'N, longitude 1°00' and 1°30'W and covers about 1 674 sq km of Sahelian savannah with a population of approximately 150 000 (Akalifa, et al., 2011; Appawu et al., 2004). Most people live in multi-family compounds of dispersed settlements separated from one another by expanse fields of farm land. The region receives approximately 850 mm of precipitation per year with monthly temperatures ranging between 18°C and 45°C (Kasasa et al., 2013). Most of the rains occur between the wet months of July and September. The rainy season, however, extends from May to October, and the rest of the months constitute the dry season.

The area has an open irrigation system which is used to flood the fields during the dry season. The irrigation system is supported by a large water reservoir in the middle of the district spanning an area of over 1860 hectares and serving 32km of main canals. There also exist numerous small water impoundments dotted throughout the district which harvest water during the rainy season for later use in the dry season. It goes without saying that the main means of livelihood in this area is subsistence farming. The irrigation system provides wet conditions excellent for mosquito breeding even during the dry season.

As can be expected malaria transmission in Kassena Nankana is perennial, but characterized by marked seasonal variation (Binka, 1994). The current entomological inoculation rate is not known, but data published more than 10 years ago reported up to 418 infective bites per person per year (Appawu, 2004). Rates of infection in children <5 years of age range from 33% during the season characterized by low transmission to 65% during the high transmission season (May – September) (Koram et al., 2000).

Kassena-Nankana is served by Navrongo Research Center located in Navrongo, Kassena-Nankana East District which runs the Navrongo Health and Demographic Surveillance System (NHDSS).

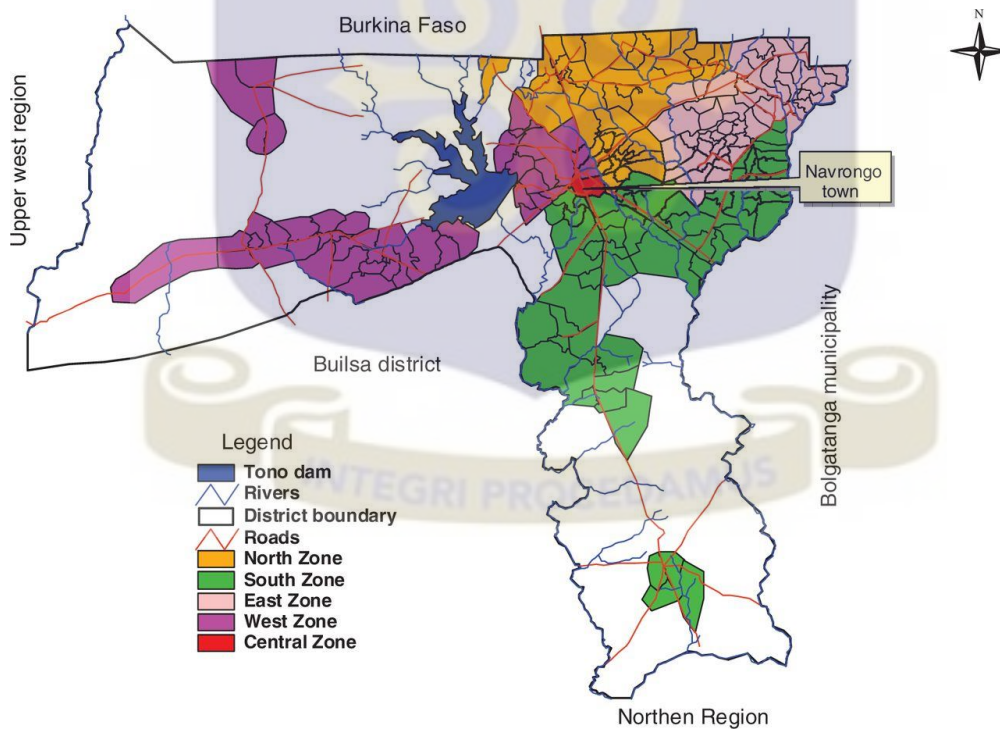


Figure 2. Map of the Kassena-Nankana district showing the district boundaries, the five zones of the NHDSS and other physical landmarks within the district (Oduro et al., 2012).

3.2.2 The Health Facilities

The study was conducted in three health facilities: War Memorial Hospital, Navrongo Central Health Center and Paga Health Center in to June, 2016, at the start of the wet season. War Memorial Hospital (the district hospital) and Navrongo Central Health Center are located in Navrongo, the district capital of Kassena-Nankana East district. War Memorial Hospital and Navrongo Central Health Center are about 2-3 kilometers apart. Paga Health Center is situated in Paga, the district capital of Kassena-Nankana West district, at a distance of about 10 kilometers from Navrongo.

War Memorial Hospital has a bed capacity of 164 and serves a population of 180,611 of the two Kassena-Nankana districts (Ghana Statistical Service, 2010). Navrongo Health Center has an average OPD attendance of 85 patients per day. Paga was recently accorded the status of a district hospital but is yet to be upgraded to that status. Currently all its operations are at health center level. War Memorial Hospital serves as its immediate referral facility. The OPD attendance at Paga averages 70 patients per day.

All three health facilities have functioning microscopy and other laboratory services. Microscopy is the main stay or routine method for parasitological diagnosis of malaria in all three. Cadres of staff performing microscopy ranges from laboratory assistant to biomedical scientist. Malaria Rapid Diagnostic Tests (RDTs) are a back-up method used only when microscopy services are not available. However, at the time the study begun Paga had had no functioning microscopy services for the past 6 months. This situation prevailed throughout the study period. During this time RDTs performed in the laboratory

were used to look for parasitological evidence of malaria infection, and the results informed doctors decision whether to treat for malaria or not.

Navrongo Central Health Center had functioning microscopy services during the entire study period except for the last three days towards the end of the study. During these last three days RDTs were used to confirm or exclude malaria, the results of which informed doctor's decision whether to treat for malaria or not. The proportion of patients seen during these last three days has been included in the analysis of prescriber adherence to RDT results.

War Memorial Hospital had functioning microscopy services during the entire study period. Patients from this facility have not been included in the analysis for prescriber adherence to RDTs results because doctor's decision to treat for malaria or not was based on microscopy results. In the interest of this study however, an RDT test was also performed on every one of these patients.

3.4 Variables

The main variable of interest, also referred to as the dependent or outcome variable were RDT positive test results among microscopically confirmed positive results (sensitivity), RDT negative test results among microscopically confirmed negative results (specificity), and the proportion of patients appropriately treated based on microscopy results.

Predictor variables were *Plasmodium* species and parasite density. Other co-variables included: cadre of health staff performing the test, self or prior treatment with antimalarials within the past 4 weeks prior to this visit, age of patient, history (reported) of fever, documented or measured fever of $\geq 37.5^{\circ}\text{C}$ on consultation, storage conditions of test kits

(temperature), product name or brand of RDTs, and whether the RDT brand is approved by WHO. Operational definitions of outcome variables are as shown in the table below.

Table 1. Outcome variables, operational definitions and how they were measured

Variable	Operational definition	Computation
Sensitivity	Percentage of RDT +ve results among samples confirmed to be malaria positive by microscopy examinations	From a 2 by 2 table
Specificity	Percentage of RDT -ve results among samples whose thick blood films are -ve by microscopy	From a 2 by 2 table
Positive Predictive value	Proportion of true +ve results among all positive samples	From a 2 by 2 table
Negative predictive value	Proportion of true -ve results among all negative samples	From a 2 by 2 table
Adherence to +ve RDT results	The proportion of +ve results for which antimalarials are prescribed	
Adherence to negative RDT results	The proportion of RDT negative results for which no antimalarials are prescribed	
Overall compliance	The overall proportion that was managed for malaria according to RDT results.	

3.5 Sampling

Out-patients presenting to health facilities whom upon consultation with a clinician he orders a parasitological test (RDT) to confirm or refute his clinical diagnosis for malaria as per WHO and Ghana Health Service Policy were serially enrolled into the study until the predetermined sample size was reached.

3.5.1 Sample size estimation

The outcome variable for estimating the sample size was the sensitivity (or specificity) of RDTs to detect malaria parasitaemia. WHO recommends that RDTs should have sensitivity and specificity of at least 95% in each local setting before they can be considered useful enough. To estimate a sensitivity (or specificity) of 95% to within 10% ($\pm 10\%$) margin of error and with 95% confidence, 18 true positive cases of malaria were required.

$$n \geq Z_{\alpha/2}^2 P(1-P)/d^2$$

Where:

n = sample size

$Z_{\alpha/2}$ = confidence level of 95% (standard value of 1.96)

p = pre-determined/desired value of sensitivity (95%)

d = desired precision ($\pm 10\%$)

Assuming a slide positivity rate of 5% (Ghana DHS, 2014) in patients presenting for a malaria test a minimum of 365 patients were to be recruited. Consideration a 10% attrition rate due to slide breakages and film wash-offs the sample size was pegged at 402.

3.5.2 Inclusion criteria

All outpatients attending the health facility during the study period, whom upon consultation with the clinician he requested a malaria rapid diagnostic test in order to parasitologically confirm malaria as is required by WHO and Ghana Ministry of Health were eligible for inclusion in the study. Such patients were those from whom informed consent had been sought and they were willing to participate in the study.

3.5.3 Exclusion criteria

Because of the difficulty of collecting sufficient blood samples from a single finger prick in infants only patients aged one year or more were recruited. Patients who did not give consent were also excluded from the study.

3.6 Data collection

3.6.1 Ethical considerations

Ethical clearance: The proposal was submitted to Ethical Review Committee of the Ghana Health Service for review and approval before commencement of the study.

Permission from study area: Permission to undertake the study was sought from the Regional Director of Health Services, Upper East Region, District/Municipal Directors of Health Services (Kassena-Nankana East and Kassena-Nankana West) and the respective Medical Superintendents of War Memorial Hospital, Navrongo Central Health Center and Paga Health Center.

Risks to participants: The study did not introduce any additional invasive or discomforting procedures to patients. Instead it took advantage of the routine procedures a patient normally undergoes when taking a malaria rapid test. Only one or two additional drops of blood were collected for malaria slide preparation

Voluntary consent: All study procedures were clearly explained to participants while obtaining informed consent. Hospital staff and study participants were assured of the confidentiality, data safety and appropriate data usage.

Benefits to participants (patients and facilities): RDT negative, microscopy positive results were communicated to the facilities as soon as they were available for their further action. The study also served as a quality assurance programme that monitored and evaluated the performance RDTs during the study period in the facilities where the study was undertaken.

3.6.2 Data collection tools and methods

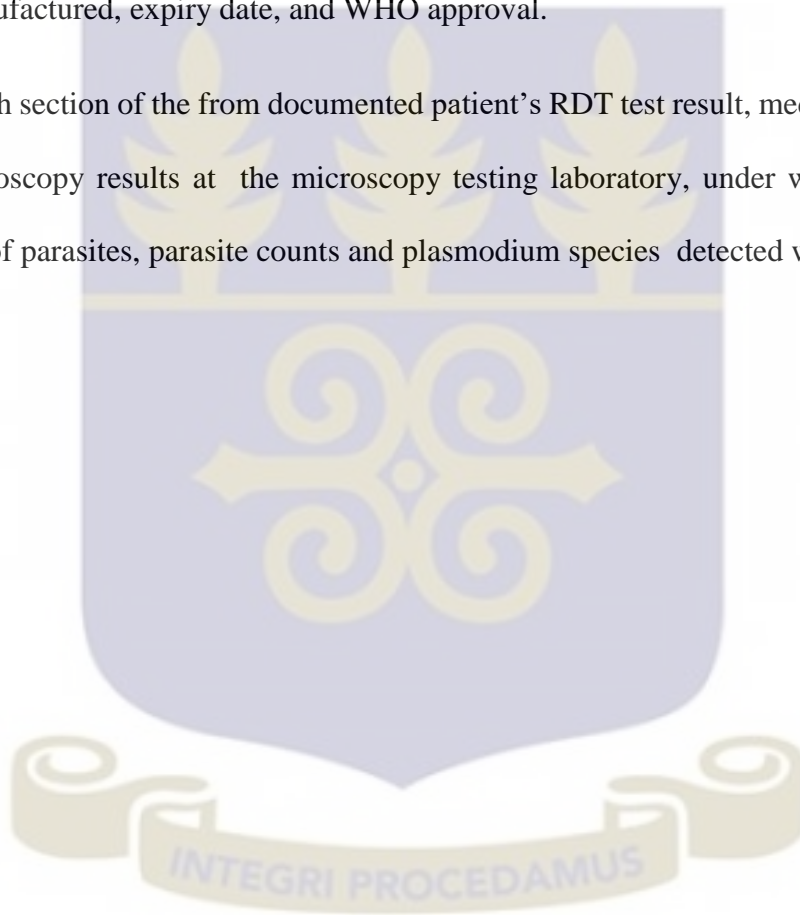
A structured form was used to collect data on factors which have the potential to affect the diagnostic accuracy of RDTs in routine settings. The first section of the form collected patient's basic demographics and clinical history (documented fever of measured body temperature $\geq 37.5^{\circ}\text{C}$ on consultation or history of fever in the previous 48 hours and history of prior treatment with an antimalarial).

The second section of the questionnaire gathered data on facility storage conditions of RDT test kits including temperature. This section of the form also gathered data on the person performing the test: cadre, years of experience with RDTs, and whether trained to do the test. A health worker was observed doing the test and was scored against manufacturer's

specified procedures. Information on both internal and external quality control procedures was sought

The third section of the form collected data on the malaria rapid test kit used at a facility, under which brand name, manufacturer, target antigens/antibodies and plasmodium species, claimed sensitivities and specificities, manufacturer's specified storage conditions, date manufactured, expiry date, and WHO approval.

The fourth section of the form documented patient's RDT test result, medicines prescribed and microscopy results at the microscopy testing laboratory, under which presence or absence of parasites, parasite counts and plasmodium species detected were recorded.



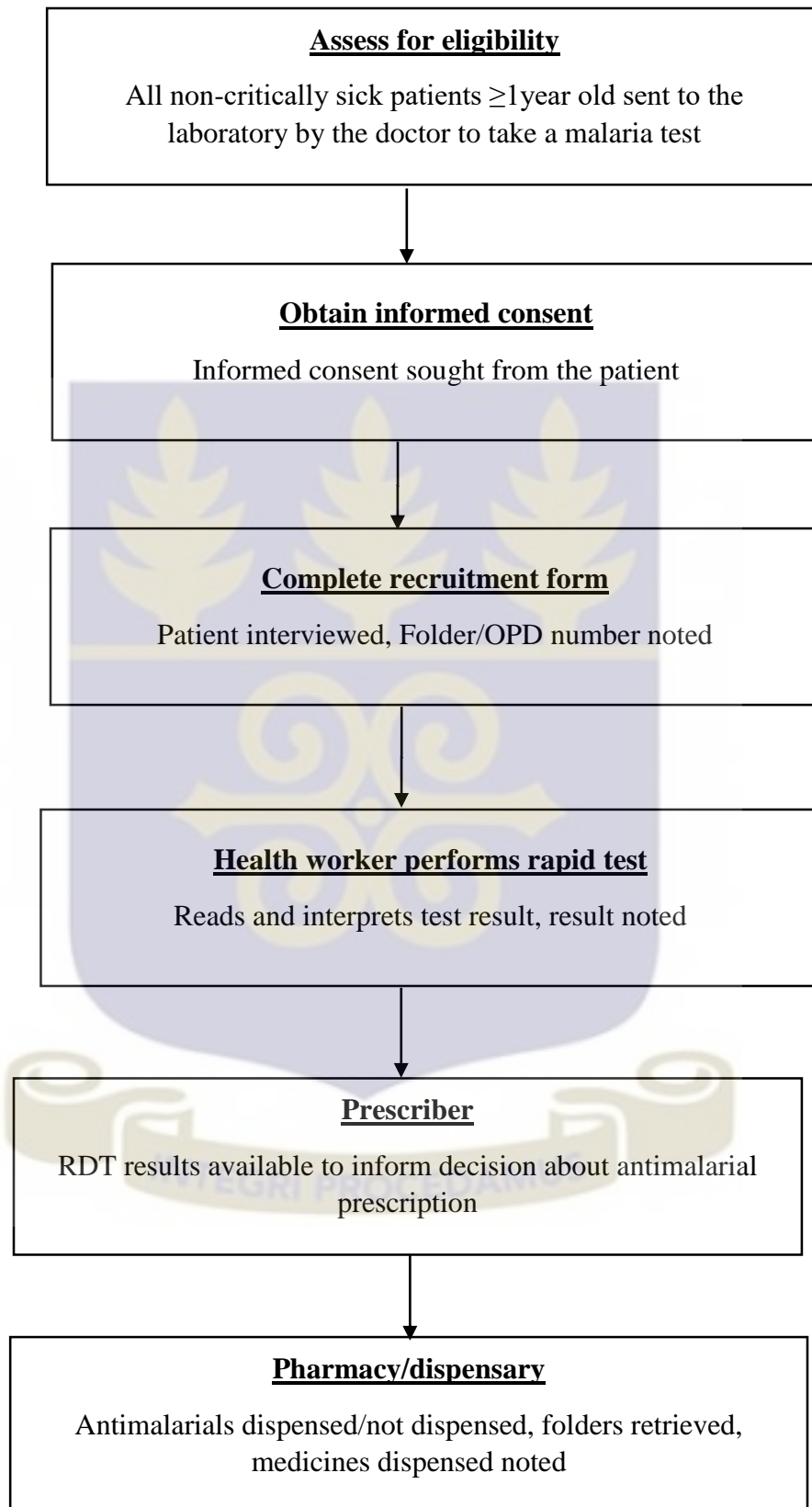


Figure 3. Flow of patients in the diagnostic accuracy of RDTs in routine settings study

Microscopy analyses

Examination of thick and thin blood films was done centrally at a District Hospital Laboratory. Thick and thin blood films were prepared from the micro EDTA blood. Both thick and thin films were prepared on the same frosted-end slide and air-dried. When sufficiently dry thin films were fixed with absolute methanol (99-100%). Dry and fixed blood films were stained with 3% Giemsa stain for 30 minutes following a standard operating procedure and examined with 100× oil objective of a light microscope.

Slides were examined by two independent expert microscopists, both blinded to the rapid diagnostic test (RDT) results and to results of each other. Thick films were used to detect parasitaemia, while thin films were used to quantify parasitaemia and identify Plasmodium species. Malaria parasite count per microlitre of blood from the thick film was estimated by counting parasites (asexual forms) against 200 white blood cells (WBC) and multiplying the count per 200 white blood cells (WBC) by 40, assuming that a microliter of blood contains 8000 WBC (Mirdha, Samantaray, & Mishra, 1997; Moody, 2002). Smears for which no parasites were detected after counting 200 WBC were scanned further until another 300 WBC were counted before being considered negative (Mirdha et al., 1997; Moody, 2002).

Discrepant results on presence or absence of parasitaemia, and a difference of parasite count of >200 asexual forms (trophozoites) per microlitre of blood between the two expert microscopists were resolved by referring to a third expert microscopist, he too blinded to the results of RDT and to those of the previous microscopists. Only asexual forms of the parasite (trophozoites) were used in the computation of parasite density. Sexual forms of the parasite (gametocytes) however, indicated malaria infection. A blood slide was

therefore, considered negative only if no trophozoites or gametocytes were seen after counting 500 WBC.

Expert microscopy was the Gold standard and was used to validate RDT results. Microscopic examinations determined *Plasmodium species* and parasitaemia. RDT results were compared to microscopy results. Sensitivity (proportion of RDT positive results among samples confirmed malaria positive by microscopy), specificity (proportion of RDT negative results among samples confirmed negative by microscopy), positive predictive value (proportion of true positives among all positive samples) and negative predictive value (proportion of true-negatives among all negative samples) were calculated as illustrated in the table below.

Table 2. Illustration of computation of parameters of a diagnostic test

		Microscopy		
		Positive (T+)	Negative (T-)	
RDT	Positive (T+)	TP	FP	(TP + FP)
	Negative (T-)	FN	TN	(TN + FN)
		P = (TP+FN)	N = (FP+TN)	Population

Sensitivity or True Positive Rate (TPR) $TPR = TP/P = TP/(TP+FN)$

Specificity (SPC) or True Negative Rate $SPC = TN/N = TN/(FP+TN)$

Precision or positive predictive value (PPV) $PPV = TP/(TP+FP)$

Negative predictive value (NVP) $NVP = TN/(TN+FN)$

False Positive Rate (FPR) $FPR = FP/N = FP/(FP+TN) = (1-SPC)$

False negative rate (FNR) $FNR = FN/(TP+FN) = (1-SENST)$

3.7 Data processing and analysis

Data were entered into Microsoft excel 2013, validated and cleaned before importing to stata version 13.0 for quantitative analysis. Chi Square test (or Fisher's exact) was used to compare if there was a significant difference between the observed values and the expected values. Logistic regression was used to determine how much each variable of interest explained the outcome (sensitivity and specificity). First a crude (univariate) analysis was performed to isolate variables explaining the outcome. Then two multivariate logistic regression models were fit to two separate sets of explanatory variables to measure the independent effect of each of those variables on the outcome (sensitivity). Only one multivariate model was run for specificity.

Microscopy, the reference method against which RDTs were compared, detected parasitaemia, identified Plasmodium species, determined parasite density and allowed the computation of sensitivity and specificity. Prescriber adherence was assessed as the proportion of patients prescribed or not prescribed antimalarials based on RDT results.

All descriptive characteristics or attributes of health facilities which have the potential to affect the diagnostic performance of RDTs (Sensitivity and Specificity), including RDTs used and cadre of staff performing RDT testing in those facilities have organized and presented in a table in the results section

CHAPTER FOUR

RESULTS

A total of 457 patients from three different health facilities were recruited into the study. All of them (100%) had complete RDT and microscopy results and were included in the analysis. Only patients from Paga Health Center and a small proportion (23%) of patients from Navrongo Health Central Health Center have been included in the analysis of prescriber adherence to RDT results.

4.1 Health facility, RDT and test operator characteristics

Health facility, RDT and test operator characteristics of the three health facilities are shown in Table 3. No characteristic with the potential to affect the performance RDTs was observed in these facilities during the time of the study.



Table 3. Health facility characteristics in the Diagnostic Accuracy of RDTs study in Kassena-Nankana districts, Ghana

Facility type	District Hospital		Health Centre
Facility name	War memorial	Navrongo Central	Paga
Health facility characteristics			
Location	Urban	Urban	Semi-urban
Average maxim temp (range)	33.2 °C (30-36)	32.7 °C (26-36)	30.1 °C (27-35)
Average minim temp (range)	31.9 °C (25-33)	31.2 °C (20-33)	29.4 °C (23-34)
RDT used	SD Bioline Mal Ag <i>Pf</i>	SD Bioline Mal Ag <i>Pf</i>	SD Bioline Mal Ag <i>Pf</i>
Target Plasmodium species	<i>P falciparum</i> only	<i>P falciparum</i> only	<i>P falciparum</i> only
Target antigens	PfHRP2	PfHRP2	PfHRP2
Manufacturer sensitivity	99.7%	99.7%	99.7%
Manufacturer specificity	99.5%	99.5%	99.5%
Specified storage conditions (temperature)	1-40 °C	1-40 °C	1-40 °C
Cadre of person performing RDT	Biomedical Scientist	Biomedical Scientist	Lab Assistant
Training	Trained	Trained	Trained
Trainer	Trained colleagues	Trained colleagues	NMCP
Experience using RDTs	3 years	3 years	4 years
RDT test procedure	available	available	Available
Quality assurance	NA	NA	Available
Supervision	*NA	NA	Available
Test operator performance score	100%	100%	100%

*Not applicable

4.2 Patient characteristics, RDT and Microscopy test results at the three health facilities

Patient characteristics varied significantly among the three health facilities, except for sex and history of fever. Overall malaria prevalence by RDTs was 10.7% (95% CI: 7.8, 13.7). Prevalence of malaria by RDTs however, varied significantly at the three health facilities ($p = 0.021$). The overall true malaria prevalence determined with microscopy was 5.0% (95% CI: 2.9, 7.1), significantly lower compared to that determined by RDTs ($p < 0.001$). Prevalence of malaria by microscopy also varied significantly among the health facilities ($p = 0.001$). The prevalence of microscopically confirmed malaria increased from District Hospital (urban) (13.0%) to urban Health Center (17.4%) to semi-urban Health Center (69.6%). *Plasmodium falciparum* was the only (100%) plasmodium species detected. Levels of parasitaemia did not vary significantly across the three health facilities (Table 4).



Table 4. Patient characteristics, RDT and Microscopy test results at three health facilities in the Diagnostic Accuracy of RDTs study in Kassena-Nankana districts, Ghana

Facility type	District Hospital	Health Centre			
Facility name	War memorial	Navrongo Central	Paga	Total	P value*
	(N = 213)	(N = 81)	(N = 163)	(N = 457)	
Patient characteristics					
Sex					
Female	146 (44.5)	66 (20.1)	116 (35.4)	328 (71.8)	0.087
Male	67 (51.9)	15 (11.6)	47 (36.4)	129 (28.2)	
Age					
1-14yrs	69 (48.6)	13 (9.2)	60 (42.3)	142 (31.1)	0.004
≥15 yrs	144 (45.7)	68 (21.8)	103 (32.7)	315 (68.9)	
History of fever					
Present	101 (53.2)	14 (7.4)	75 (39.5)	190 (41.6)	<0.001
Absent	112 (42.0)	67 (25.1)	88 (33.0)	267 (58.4)	
Confirmed fever					
≥37.5 C	17 (32.10)	2 (3.8)	34 (64.2)	53 (11.6)	0.364
<37.5	45 (42.1)	6 (5.6)	56 (52.3)	107	
Treated for malaria in the past 4 weeks					
Treated	21 (35.0)	8 (13.3)	31 (51.7)	60	0.010
Not treated	187 (49.6)	71 (18.8)	119 (31.6)	377	
RDT & Microscopy test results					
RDT Pos	14 (28.6)	10 (20.4)	25 (51.0)	49 (10.7)	0.021
RDT Neg	199 (48.8)	71 (17.4)	138 (33.8)	408 (89.3)	
Microscopy Pos	3 (13.0)	4 (17.4)	16 (69.6)	23 (5.0)	0.001
Microscopy Neg	210 (48.4)	77 (17.7)	147 (33.9)	434 (95.0)	
Plasmodium species					
<i>P falciparum</i>	3 (13.0)	4 (17.4)	16 (69.6)	23	
Non-falciparum	0 (0.0)	0 (0.0)	0 (0.0)	0	
Parasitaemia					
≤100/μL	1 (14.3)	1 (14.3)	5 (71.4)	7	1.000
>100/μL	2 (12.5)	3 (18.8)	11 (68.8)	16	

*Statistical testing using chi2 test (or Fisher's exact)

†Number in parentheses are row percentages

4.3 Association between patient characteristics and RDT and Microscopy test outcomes

Except for confirmed fever (body temperature ≥ 37.5) ($p = 0.022$) all other patient characteristics were not significantly associated with a positive RDT result. On the other hand age of < 15 ($p = 0.025$), history of fever ($p = 0.005$) and confirmed fever or measured body temperature of ≥ 37.5 °C on consultation ($p = 0.001$) were associated with a positive microscopy result (Table 5).



Table 5. Patient characteristics and RDT and Microscopy test outcomes

Patient characteristics	RDT		Total	P value*	Microscopy		Total	P value*
	Pos (%)	Neg (%)			Pos (%)	Neg (%)		
Sex								
Female	36 (11.0)	292(89.0)	328	0.780	18 (5.5)	310 (94.5)	328	0.478
Male	13 (10.1)	116 (90.0)	129		5 (3.9)	124 (96.1)	129	
Age								
1-14yrs	18 (12.7)	124 (87.3)	142	0.365	12 (8.4)	130 (91.6)	142	0.025
≥15 yrs	31 (9.8)	284 (90.2)	315		11 (3.5)	304 (96.5)	315	
History of fever								
Present	24 (12.6)	166 (87.4)	267	0.266	16 (8.4)	174 (91.6)	190	0.005
Absent	25 (9.4)	242 (90.6)	190		7 (2.6)	260 (97.4)	267	
Confirmed fever								
≥37.5 C	12 (22.6)	41 (77.4)	53	0.022	10 (18.9)	43 (81.1)	53	0.001
<37.5	10 (9.4)	97 (90.7)	107		4 (3.7)	103 (96.3)	107	
Treated for malaria in the past 4 weeks								
Yes	5 (8.3)	55 (91.7)	60	0.447	2 (3.3)	58 (96.7)	60	0.471
No	44 (11.7)	333 (88.3)	377		21 (5.6)	356 (94.4)	377	

*Statistical testing using Ch2 (or Fisher's exact)

4.4 Diagnostic accuracy of malaria rapid tests (RDTs) in routine field settings

SD Bioline Malaria Ag *Pf* was the only RDT used and evaluated in this study. The overall sensitivity was 69.6% (CI: 67.8 - 71.4) and the overall specificity was 92.4% (CI: 89.9 - 94.9) (Figure 4). The corresponding false negative rate and false positive rate were 30.4% and 7.6% respectively. Both sensitivity and specificity varied across sites with highest sensitivity of 75% at Paga Health Center and lowest (50%) at Navrongo Central Health Center (Table 6). At all facilities however, the sensitivity was far lower than the required 95%. Specificity ranged from 89.6% at Navrongo Central Health Center to 94.3% at War Memorial District Hospital. Navrongo Central Health Center performed the poorest in both sensitivity and specificity. Because of the poor overall and facility-specific sensitivities and good overall and facility specific specificities the positive predictive value and negative predictive value were low (32.7%) and high (98.3%) respectively (Figure 4).



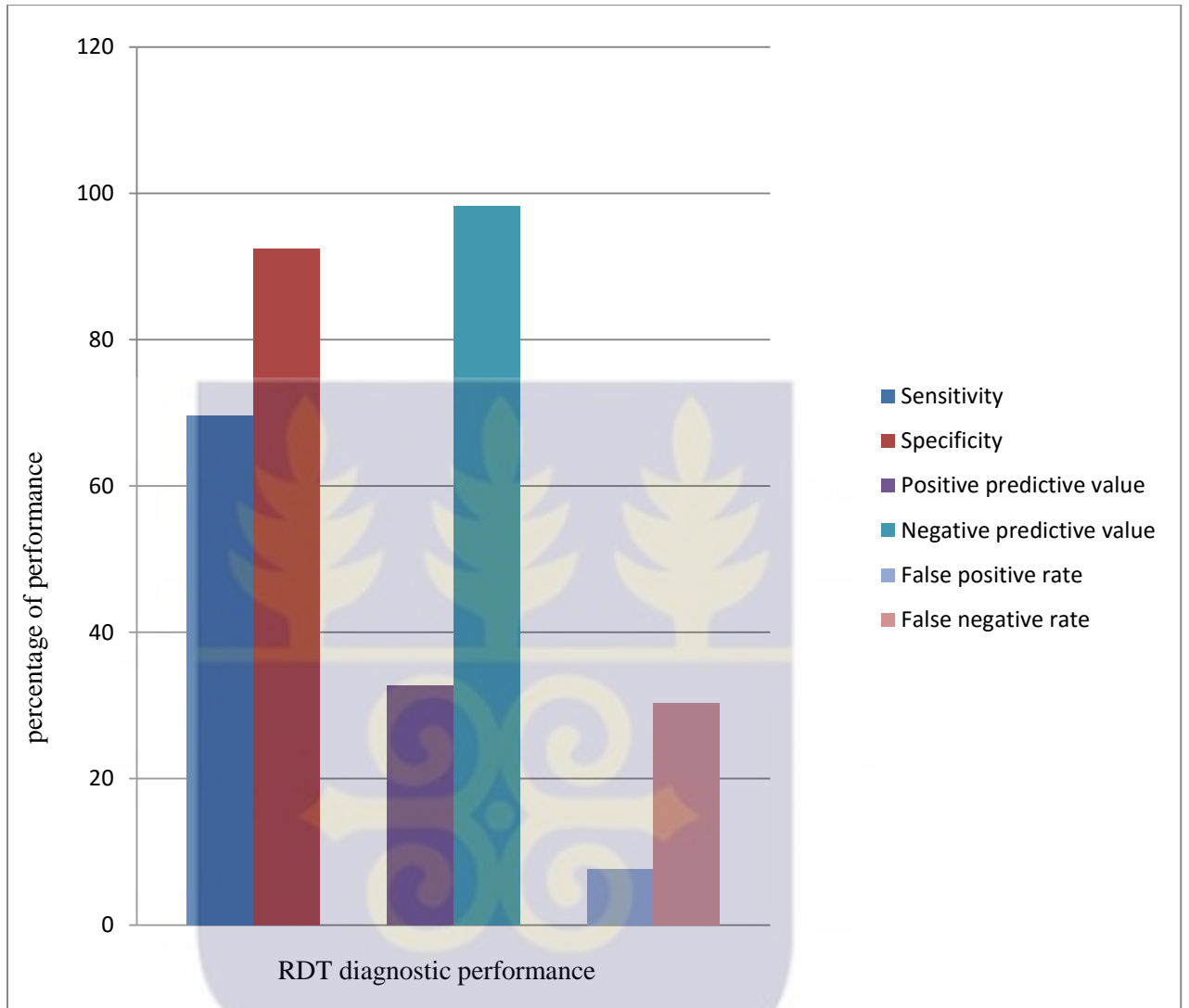
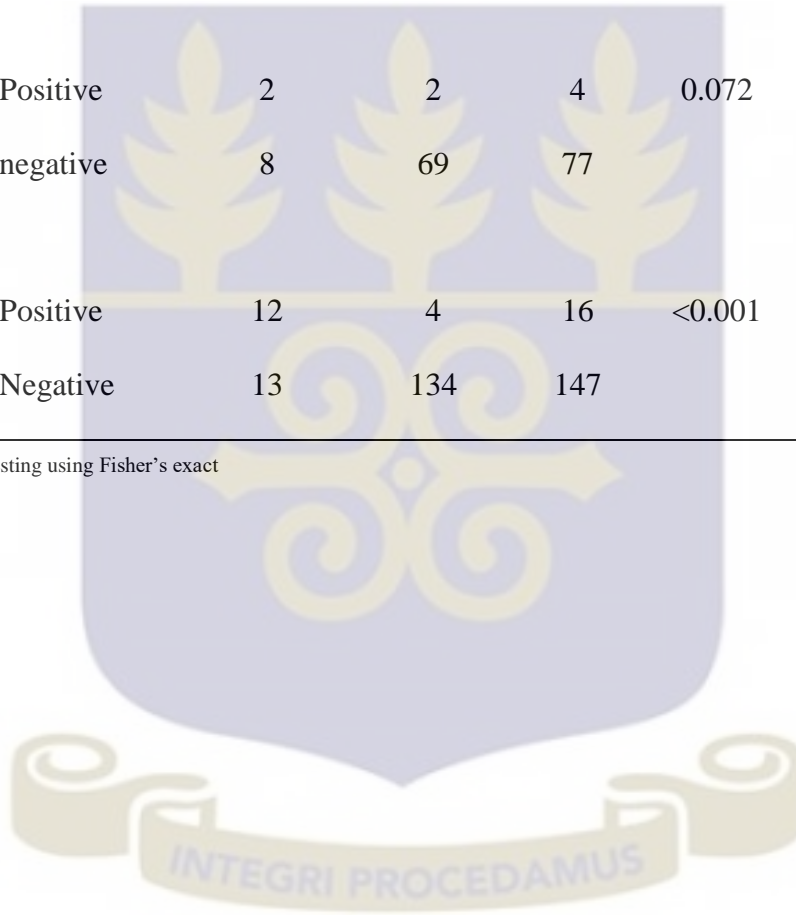


Figure 4. Diagnostic performance of malaria rapid diagnostic tests (RDTs) in routine field settings

Table 6. Sensitivity and Specificity of RDTs at three health facilities where the study was undertaken

Facility	Microscopy	RDT		Total	P value*	Sensitivity	Specificity
		Positive	Negative				
War Memorial	Positive	2	1	3	0.012	66.7%	94.3%
	Negative	12	198	210			
Navrongo H/C	Positive	2	2	4	0.072	50.0%	89.6%
	negative	8	69	77			
Paga H/C	Positive	12	4	16	<0.001	75.0%	91.2%
	Negative	13	134	147			

*Statistical testing using Fisher's exact



4.5 Predictors of diagnostic accuracy of malaria rapid diagnostic tests (RDTs)

Given the low overall and facility-specific sensitivity, but coupled with high overall and facility-specific specificity of the RDT tested, potential factors for this phenomenon were explored. Using a logistic regression patients age (1-14yrs Vs. ≥ 15 yrs), history of fever, confirmed fever (body temp ≥ 37.5 °C Vs. < 37.5 °C), Parasitaemia ($\leq 100/\mu$ Vs. $> 100/\mu$), cadre of test operator (Biomedic Vs. Lab assistant) (0.003) and facility type (District Hospital Vs, Health Center), were significantly associated with sensitivity in a univariate model (Table 7). Sensitivity was significantly higher in 1-14yrs age group ($p = 0.003$), when patients complained of fever ($p = 0.003$), when fever was confirmed ($p = 0.002$), at the health center ($p = 0.015$) but significantly lower when parasitaemia was less than 100 parasites per microlitre of blood ($p = 0.003$), and when testing was carried out by a Biomedic ($p = 0.003$). In a multivariate analysis, however, confirmed fever (body temp ≥ 37.5 °C) significantly predicted high RDT sensitivity ($p = 0.027$) in model 1 whilst parasitaemia of ≤ 100 parasites per microliter of blood significantly ($p = 0.009$) predicted low sensitivity in mode 2.



Table 7. Predictors of malaria rapid diagnostic test (RDT) Sensitivity (RDT positive, expert microscopy positive)

	Sensitivity n/N (%)	*Unadjusted OR (P value)	*Adjusted OR Model 1 (P value)	*Adjusted OR Model 2 (P value)
Age				
1-14 yrs	11/12 (91.8)	5.2 (0.003)	3.7 (0.125)	4.7 (0.353)
≥15 yrs	5/11 (45.5)	Reference	Reference	
History of fever				
Present	13/16 (81.3)	6.4 (0.003)	1.0 (0.995)	
Absent	3/7 (42.9)	Reference	Reference	
Confirmed fever				
≥37.5	10/10 (100.0)	8.1(0.002)	6.1 (0.027)	
<37.5	3/4 (75.0)	Reference	Reference	
Treated for malaria in the past 4 weeks				
Yes	1/2 (50.0%)	0.4 (0.391)		
No	15/21 (71.4)	Reference		
Parasitaemia				
≤100/μL	1/7 (14.3))	0.01 (0.003)		0.02(0.009)
>100/L	15/16 (93.8))	Referent		
Cadre				
Biomedic	4/7 (57.1)	0.17 (0.003)	1.1 (0.930)	
Lab assistant	12/16 (75.0)	Reference	Reference	
Facility type				
Health center	14/20 (70.0)	6.4 (0.015)	5.5 (0.222)	3.6 (0.907)
District hospital	2/3 (66.7)	Reference	Reference	

*Logistic regression model

Specificity was found to be significantly affected by confirmed fever (body temp ≥ 37.5 °C Vs. < 37.5 °C), cadre of test operator (Biomedic Vs. Lab assistant) and facility type (District Hospital Vs, Health Center) (Table 8). Specificity was significantly lower at the health center ($p = 0.002$) and in patients with confirmed fever (body temp ≥ 37.5 °C ($p = 0.040$), but significantly high ($p = 0.008$) when RDT testing was done by a biomedical scientist in a univariate model. In a multivariate model however, only confirmed fever (body temp ≥ 37.5 °C) was found to be a significant predictor of specificity. Specificity was significantly lower ($p = 0.035$) in patients with confirmed fever (body temp ≥ 37.5 °C)

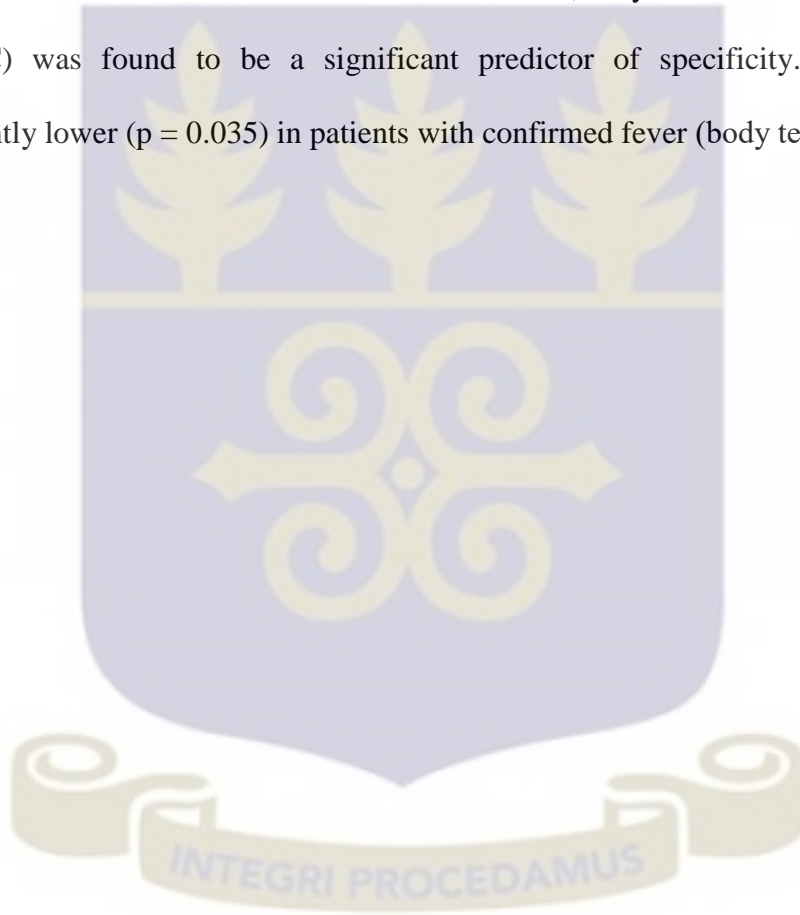


Table 8. Predictors of malaria rapid diagnostic test (RDT) Specificity (RDT negative, expert microscopy negative)

	Specificity n/N (%)	Unadjusted odds ratio *(P value)	Adjusted odds ratio *(P value)
Age			
1-14yrs	123/130 (94.6)	0.9 (0.622)	1.0 (0.961)
≥15yrs	278/304 (91.5)	Reference	Reference
History of fever			
Present	163/179 (93.1)	0.7 (0.186)	1.8 (0.398)
Absent	238/259 (91.9)	Reference	Reference
Confirmed fever			
≥37.5	41/43 (95.4)	0.4 (0.040)	0.3 (0.035)
<37.5	96/103 (93.2)	Reference	Reference
Treated for malaria in the past 4 weeks			
Treated	54/58 (93.1)	1.4 (0.484)	7.3 (0.067)
Not treated	327/356 (91.9)	Reference	Reference
Cadre			
Biomedic	267/287 (93.0)	2.1 (0.008)	0.8 (0.815)
Lab assistant	134/147 (91.2)	Reference	Reference
Facility type			
Health center	203/224 (90.6)	0.4 (0.002)	0.3 (0.196)
District hospital	198/210 (94.3)	Reference	Reference

*Statistical testing using logistic regression

4.6 Prescriber adherence to RDT results

One hundred percent (100%) prescriber adherence to both positive and negative RDT results was observed at Paga and Navrongo Central Health Centers (Table 9). However, for those patients who were treated for malaria based on RDT results less than half (43%, 12/28) were appropriately treated. For patients who were not given antimalarials 3% (4/154) had malaria. Overall 89% (162/182) were appropriately managed for malaria whilst 11% (20/182) were inappropriately managed. Since we are certain that for all these patients a malaria test was ordered by their physician because they had met some clinical criteria for suspecting malaria then in this study RDTs achieved an overall level of 89% as regards to appropriate management of suspected malaria.

Table 9. Prescriber adherence to malaria rapid diagnostic test (RDT) results

RDT test results	Microscopy		Treatment for malaria	
	True +ve	True -ve	Treated	Not treated
RDT positive	12	16	28	0
RDT negative	4	150	0	154
Total	16	166	28	154

CHAPTER FIVE

DISCUSSIONS

5.1 Prevalence of parasitaemia and transmitting plasmodium species

This study was undertaken in Kassena-Nankana districts of the Upper East region, Northern Ghana. The objective of the study was to evaluate the diagnostic performance of Malaria Rapid Tests (RDTs) in routine field settings. Data was collected at three health facilities: War Memorial Hospital and Navrongo Central Health Center in Kassena-Nankana East and Paga Health Center in Kassena-Nankana West. Outpatients presenting to the health facility for whom their doctor had requested a malaria test were serially enrolled into the study. A capillary blood sample was collected in a micro-EDTA tube for routine RDT testing and blood film for microscopy. Expert microscopy was the reference method against which RDTs were compared.

The prevalence of parasitaemia by the reference method was 5%. The prevalence was lower compared to what previous studies have documented in the area. (Koram et al., 2003) reported a prevalence of 22% during the dry-low transmission season and 61% during the wet-high transmission season in the same district. Another study undertaken in another district in Northern Ghana reported parasitaemia of 53% in children aged 6 months to 9 years (Danquah et al., 2010). The result however, agrees with most recent studies which have reported that the incidence of malaria in sub-Saharan Africa has substantially declined (Cibulskis et al., 2016; WHO, 2015). Ghana for instance, has recently been transformed from a malaria hyper-endemic country to a meso-endemic country. During the low transmission season (December – June) malaria incidence goes further down to an extent

that War Memorial Hospital, for example, could go for a day without prescribing an antimalarial.

Plasmodium falciparum was the only plasmodium species that was transmitting and infecting people at the beginning of the high transmission season. This finding agrees with other studies which have extensively documented *Plasmodium falciparum* as the most prevalent (up to 100%) species in Ghana (Asante et al., 2011; Manyando et al., 2014; WHO, 2015). However, one previous study, also conducted in Northern Ghana during the low transmission season (January – April) reported the existence of other plasmodium species including *P. malariae*, *P. ovale*, and a lesser prevalence of *P. falciparum*. Danquah et al., (2010) reported a microscopically visible parasitaemia of 61% for *P. falciparum*, 8.5% for *P. malariae* and 4.4% for *P. ovale*. Dinko et al., (2013) and Oguike et al., (2014) also reported some marginal prevalence of *P. ovale* and *P. malariae* in other regions of Ghana, including middle belt. The explanation to the discrepancy in the findings could be that due to recent gains in malaria control malaria transmission has declined significantly such that it is mainly *P. falciparum* which persists the low transmission season to cause clinical malaria at the beginning of the high transmission season.

5.2 Predictors of diagnostic accuracy malaria rapid tests (RDTs) in routine settings

SD Bioline Malaria Ag Pf is the only RDT that was evaluated in this study because it was the only one that was in use in all the health facilities where the study was undertaken at the time the study was being undertaken. SD Bioline Malaria Ag P detects the histidine-rich protein-2 (PfHRP-2) of *P. falciparum* only. RDTs which detect HRP-2 are usually associated with good sensitivities, even at low levels of parasitaemia (Maltha et al., 2014;

WHO, 2014). In this study, however, the sensitivity SD Bioline Malaria Ag Pf was 69.6%, far lower than what other published studies have reported. Few studies have reported sensitivities as low as this (Endeshaw et al., 2010; McMorrow, 2010; Muhindo et al., 2012). One study evaluating the diagnostic utility of RDTs in an area with declining malaria burden in north-eastern Tanzania found a much lower (63.4%) sensitivity with RDTs (Ishengoma et al., 2011) while another study in Nigeria found a shockingly lower (44.3%) sensitivity with RDTs in infants (Oyeyemi, 2016). Most studies have recorded good sensitivities with RDTs, some as high as 100% (Abeku et al., 2008; Azikiwe et al., 2012; Baiden, 2012; Chinkhumba et al., 2010; Mills et al., 2010; Tarimo, 2015). All these studies were undertaken in the high transmission season and most of them reported poor specificities during this time, some as low as 39% with SD Bioline Malaria Ag Pf. In contrast to those studies this study found good specificity (92.4%) with RDTs. This contradiction suggest that sensitivity and specificity of RDTs are influenced by intensity of transmission. During the high transmission season sensitivity peaks while specificity crashes. During the low transmission season specificity improves significantly while sensitivity wobbles.

Because of the worryingly low sensitivity but encouraging specificity factors associated with the phenomena were explored. Both biological and operational factors that could have resulted in low sensitivity and good specificity in this study were investigated. Parasitaemia of ≤ 100 parasites per microliter of blood and if the test was performed by a biomedical scientist were significantly associated with low sensitivity. After adjusting for other variables cadre of the test operator too became insignificant.

It was not possible to assess all predictors of sensitivity in a single unified multivariate model because of the limited number of observations in the two categories of parasitaemia. A few high sensitivity and low sensitivity predictors observed in the crude analysis were thus selected and reassessed in a second model. Only parasitaemia of ≤ 100 parasites per microliter of blood retained its status as significant lone predictor of low sensitivity. Numerous studies undertaken in different settings have unanimously reported low parasitaemia as the most important and independent biological predictor of low sensitivity (Abeku et al., 2008; Endeshaw et al., 2010; Fogg et al., 2008; Kilian et al., 2000; McMorro et al., 2010; Muhindo et al., 2012; Oyeyemi et al., 2016; Tahar et al., 2013). This study corroborates these findings. Other factors such as intrinsic properties of the RDTs such the inter-lot variability, failure to maintain a cold chain (i.e. exposure to extreme temperatures $> 40^{\circ}\text{C}$ for more than 7 days, often during transportation) (WHO, 2014), genetic variability in the PfHRP-2 gene (Lee et al., 2006a, 2006b; Maltha et al., 2014) and deletion of the PfHRP-2 gene (Gamboa et al., 2010; Wurtz et al., 2013) have been implicated in the low sensitivity of RDTs. However, none of these factors appear to have played a role in the worryingly low sensitivity observed in this study since sensitivity was very good (93.8%) in parasitaemia $> 100/\mu$ but dropped sharply to 14.3% when parasitaemia was $\leq 100/\mu$, suggesting that parasitaemia alone is to blame.

In an evaluation for specificity cadre of test operator (biomedical scientist) showed a significant crude association with high specificity whereas health facility (health center) and confirmed fever (measured body temperature of $\geq 37.5^{\circ}\text{C}$) demonstrated significant negative prediction for specificity on a crude scale. After adjusting for other variables only confirmed fever (measured body temperature of $\geq 37.5^{\circ}\text{C}$) was significantly associated with

low specificity, with the association becoming more profound than in the crude analysis. No plausible biological explanation was found for this rather bizarre association which if truly exists then it is yet to be documented.

Previous studies have reported an association between previous treatment with antimalarials and low specificity (Chinkhumba et al., 2010). Surprisingly this study did not find any association between the two. This may be because the investigators used a cut-off period of two weeks for previous treatment. This study therefore, contradicts those which have reported that PfHRP-2 persists at detectable levels until five weeks (37 days) after successful treatment and clearance of parasitaemia (Kyabayinze, 2008; McMorro et al., 2010; Tahar et al., 2013), but corroborates those studies which have reported two weeks as the maximum duration until which PfHRP-2 can be detected following successful treatment and clearance of parasitaemia (Swarthout, 2007).

However, it is worth noting that studies which have reported 37 days were conducted in the high transmission seasons during which malaria attacks are usually accompanied by high loads of parasitaemia and corresponding high antigen titers of PfHRP-2 which takes a relatively longer time to clear after successful treatment and clearance of parasitaemia. Kyabayinze et al., (2008) found that the duration taken to clear persisting PfHRP-2 varied significantly depending on pre-treatment levels of the antigen. In patients with parasitaemia of $>50,000/\mu\text{L}$, the mean duration of persistent antigenicity was 37 days compared to 26 days for parasitaemia less than $1,000/\mu\text{L}$.

Another study in East Africa reported an association between age of patient and specificity (Abeku et al., 2008). Specificity was significantly higher in the older age group (≥ 15 years) compared to the younger age group (< 15 years) and that it improved towards the end of the

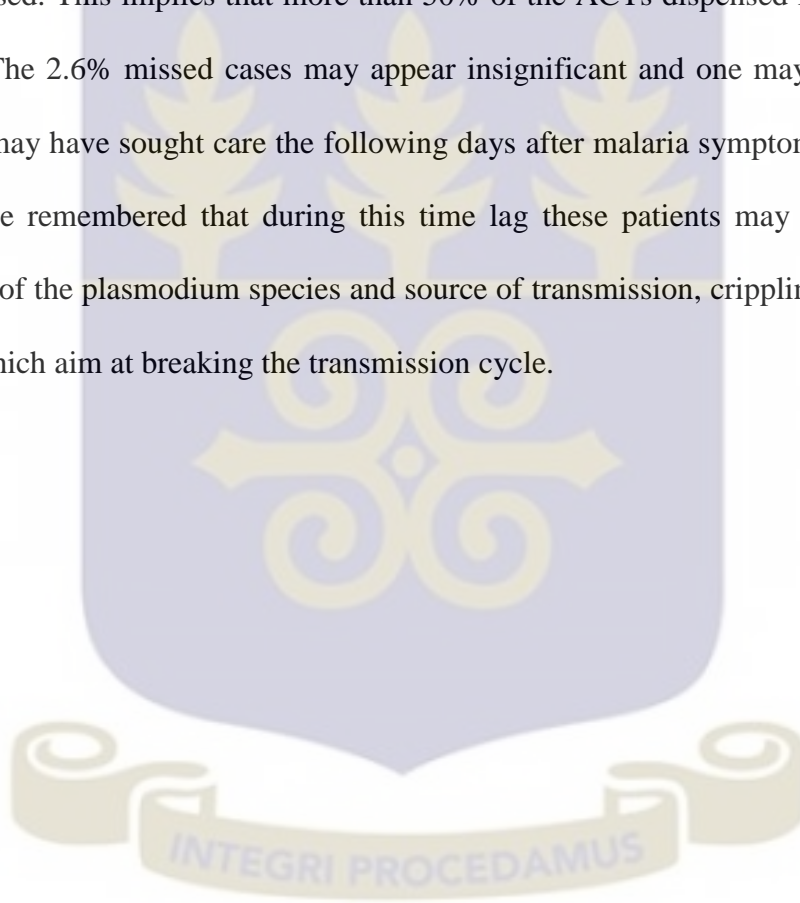
high transmission season. This study did not find any significant association between high specificity and being 15 years of age or older. The discrepancy may be due to the fact that the East African study was undertaken during the high transmission season during which frequent malaria attacks are common. During this time adult individuals with mature and robust immunity are able to quickly clear PfHRP-2 antigens with their anti-PfHRP-2 antibodies following successful treatment and clearing of parasites compared to younger individuals whose immune systems are still developing. During the low transmission however, frequent malaria attacks are rare such that regardless of age every individual has ample time to clear PfHRP-2 antigens following effective treatment and clearance of parasitaemia before the next episode of malaria.

5.3 Prescriber adherence to RDT results

The phenomenon of prescriber non-adherence to RDT results and prescribing antimalarials to patients without parasitological evidence of malaria is well documented (Ansah et al., 2010; Chinkhumba et al., 2010; Reyburn et al., 2007; Skarbinski et al., 2009; Zurovac et al., 2008). In most of these instances non-adherence was to negative RDT results leading to over-diagnosis and unnecessary treatment for malaria. On the contrary to most published data this study found a one-hundred-percent (100%) adherence to both negative and positive RDT results. This is good news for Ghana. This change may be attributed to current malaria interventions by the Ghana Health Service through the National Malaria Control Programme which has been championing the latest universal “Test and Treat” strategy for management of malaria since its inception from WHO in 2010. The National Health Insurance Scheme may also have contributed to this positive behavior change since

it has declined to pay for any patient treated for malarial without parasitological evidence of malaria.

One hundred percent prescriber adherence observed in this study did not however, lead to 100% appropriate management of patients with suspected malaria. Only 43% of all patients treated for malaria in this study actually had malaria. A further 2.6% true malaria cases were missed. This implies that more than 50% of the ACTs dispensed in this study were wasted. The 2.6% missed cases may appear insignificant and one may argue that these patients may have sought care the following days after malaria symptoms got worse. But it must be remembered that during this time lag these patients may have served as a reservoir of the plasmodium species and source of transmission, crippling malaria control efforts which aim at breaking the transmission cycle.



CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has revealed that clinical malaria at the beginning of the high transmission season is characterized by low levels of parasitaemia with *Plasmodium falciparum* as the only species causing clinical malaria. It is thus imperative to procure and deploy RDTs which will detect *P. falciparum* at substantially lower levels of parasitaemia observed in this study. But as at now there is no available RDT on the market that can detect lower parasitaemia better than those targeting PfHRP2 such as the SD Bioline malaria Ag Pf evaluated in this study.

The diagnostic accuracy of RDTs evaluated in this study was unsatisfactory. The sensitivity was far lower than the manufacturer's claim, and does not meet optimum levels of sensitivity for field settings stipulated by WHO. Although specificity was optimal it fell short of the manufacturer's specification and what WHO considers optimal for each specific setting. The poor sensitivity observed is wholly attributable to low levels of parasitaemia, but which are clinically significant. Parasitaemia is influenced by transmission intensity, implying that RDTs would give varying levels of sensitivities and specificities during the low and high transmission seasons. Much of this problem could be addressed by the manufacturer, but malaria endemic countries and sub regions/districts have a role to play to ensure that RDTs deployed in their specific settings perform satisfactorily.

Previous studies have reported a significant association between the cadre of the test operator and sensitivity and specificity of RDTs. Diagnostic performance of RDTs varied between test operators and between facilities, with lower cadres of health workers at lower level facilities of the health system performing the least (Chinkhumba et al., 2010). In this study RDT testing was carried out by qualified trained and experienced laboratory technicians, and in facilities with capacity to perform routine microscopy, and yet the sensitivity of RDTs was worryingly low. The diagnostic performance of RDTs may be even lower in lower level health facilities of the health system where RDT testing is performed by lowest cadres of health workers. The phenomenon may also exist in higher facilities where RDT testing is done by lower cadres of health staff who are not appropriately trained and supervised. There is need to investigate the performance of RDTs in those settings.

Prescriber adherence to RDT results was excellent. But this level of adherence cannot be generalized for other facilities because War Memorial Hospital and the surrounding health centers (Navrongo Central, Paga) is one of satellite sites for the National Malaria Control Programme where new malaria interventions are first introduced and championed. Usually such facilities are prioritized and receive sufficient training and supportive supervision which may have contributed to behavior change giving the observed levels of adherence. However, the result does demonstrate that the universal ‘Test and Treat’ strategy which hinges on prescriber adherence to RDT results is attainable.

Successful implementation of the ‘Test and Treat’ strategy alone will however, not lead to appropriate management of malaria. Eleven percent (11%) of patients in this study, which consists of 20% missed cases of malaria and 80% treated for malaria but did not have

malaria were inappropriately managed. The latter had other equally life threatening conditions which may have been missed. All attributable to challenges with the diagnostic method (RDTs) used, annulling the well-founded claim that RDTs are not achieving their full potential because prescribers do not apply treatment according to test results.

RDTs have on the overall significantly improved the targeting of antimalarials. However, with the sustained decline in the incidence of malaria resulting in low but clinically significant parasitaemia RDTs are fast losing their diagnostic utility. Consequently the Test and Treat strategy is fast becoming obsolete. Further control/reductions beyond current incident levels of malaria call for improvements in diagnostic and management guidelines

6.2 Recommendations

This study has shown that RDTs are no longer sufficient to be a stand-alone method upon which to wholly base clinical decision. There is need to complement RDTs with better performing diagnostic technologies, while at the same time calling for more studies to replicate the findings of this study. Microscopy is readily available. There is just need for some small investment in human resource capacity and infrastructural development so that every health facility should have at least a close-by facility where microscopy services are available to which they can always refer.

There is Need for setting-specific malaria management guidelines which take into account transmission dynamics, to be used simultaneously with the “Test and Treat” strategy, rather than total dependence on RDTs as is the case at the moment. For instance clinicians could

be retrained to interpret RDT results cautiously based on the clinical presentation of the patient and the transmission intensity of malaria (low or high) and encouraged to refer when an advanced diagnostic technology is needed to confirm or refute a suspicious RDT result.

There is need to develop a robust surveillance system to monitor the performance of RDTs over time as malaria incidence continues to decline in many malaria endemic countries and sub regions due to effective interventions taking shape in those countries. The information garnered would inform selection of RDT products which meet the current epidemiology of malaria in those settings. The data from the surveillance system would also inform new malaria management guidelines that could be used concomitantly with the Test and Treat strategy. Such information could also be fed back to manufacturers of RDTs to improve upon their products so that they meet field settings.

This study was undertaken in just one setting of the many different malaria transmission settings which exist in Ghana. There is need for a larger study to ascertain the diagnostic performance of RDTs in routine field settings across Ghana during both the high and low transmission seasons.



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APPENDICES

CASE RECORD FORM FOR DATA COLLECTION

FOLDER/OPD No.:

Study title: Diagnostic accuracy of malaria rapid tests in routine settings in Kassena-Nankana District of Northern Ghana: Implications for the test and treat policy

1. Patient ID.....
2. Date (dd/mm/yyyy)
3. Facility name:
4. Type of facility: District hospital Health center CHIPS Compound

Section A: Patient characteristics

1. Sex: Male Female
2. Age (in completed years):
3. History of fever: Present Absent Temperature
4. Prior treatment with antimalarial in the past **4 weeks**: Yes No

Section B: Health facility characteristics

1. Room temperature: Min....., Max.....
2. Test operator: ID No..... Cadre
3. Trained: Yes No Trained by
4. Years of experience with RDTs.....
5. Availability of RDT test procedure/interpretation of results/.....
6. Test performance score (correctly performs all steps in test procedure)

7. Availability of external/internal quality assurance: Available
(describe)..... Not available
8. Any form of supervision: Yes (describe) No How often

Section C: Malaria rapid test kit

1. Product/Brand name
2. Manufacturer
3. Plasmodium species targeted
4. Target antigens: pfHRP-2/3 pLDH P. aldolase
5. Specified storage conditions (temperature)
6. Indicated sensitivity 7. Specificity
8. Meets WHO procurement criteria: Yes No
9. Date manufactured Expiry date

Section D: RDT test outcome and patient management

1. RDT test result: Positive Negative
2. Diagnosis/treatment: antimalarial dispensed Not dispensed

Section E: Microscopy

1. Microscopy results: Positive (asexual/sexual forms) Negative
2. Plasmodium species: *P. falciparum* *P. vivax* *P. malariae* *P. ovale*
3. Parasite count/ μ L

INFORMED CONSENT FORM FOR STUDY PARTICIPANTS

Study title: Diagnostic accuracy of malaria rapid tests in routine settings in

Kassena-Nankana District of Northern Ghana: Implication for the test and treat policy

Introduction

My name is Alfred B. Kayira. I am a graduate student with the University of Ghana pursuing a Master of Public Health. We are conducting a study on diagnostic accuracy of malaria rapid tests in our setting. I would like to invite you to participate in the study because following consultation with your doctor you have been asked take a malaria rapid test and you are here just about to take the test.

Malaria remains a major public health problem claiming close to a million lives per year around the world. Ninety percent of these deaths occur in sub-Saharan Africa, including Ghana. Children, pregnant women and the elderly are particularly vulnerable. The key step in improving treatment outcomes in patients with malaria is prompt and accurate diagnosis so that a patient gets a timely and appropriate treatment. This study seeks to find out whether rapid tests used in our facility are working very well and so we want to compare the RDT results and those obtained from microscopy which is the reference test method. It goes further to explore factors that could be associated with the test results, if there are any. The findings and recommendations from the study will be useful to various authorities including the Ghana Health Service to improve the diagnostic performance of RDTs in our setting.

Study procedure

You (or your child, ward) are cordially invited to take part in this study. We will ask you questions about your age, what you have been feeling (signs and symptoms) in the past two days, whether you sought treatment elsewhere before coming to this facility, and the type of medication you were given. Then one or two drops of blood will be collected from the same finger prick site you had for your RDT test. These drops of blood will be used to make a blood film on a glass slide. The film will be examined by microscopy at reference laboratory and your true malaria status will be determined.

Voluntariness

Taking part in this study is absolutely voluntary. You have every right to refuse to participate or let your child/ward participate. If you (or your child/ward) chose to refuse you it will not in any way affect the quality of care you will receive for your illness.

Withdraw

If you choose to participate (or let your child/ward) participate in this study, you have the right to withdraw from it at any point in time. That too will not in any way affect the quality of care you would receive.

Risks and benefits of the study

There are no direct and immediate benefits to you (or your child/ward) for your participation in this study. We also do not anticipate any direct harm to you (or your child/ward) except that an additional one or two drops of blood will be taken for the preparation of the blood smear. However, we are hopeful that by your (or his/her) participation in this study, you (or he/she) will be helping us provide national, regional and

international health decision makers with evidence on the diagnostic performance of malaria rapid tests. In the event that your RDT result was negative and did not get antimalarials but microscopy results turns out to be positive the results shall be communicated to the health facility as soon as possible so that they can follow up on you if the doctor thinks it is necessary.

Compensation

You (or your child/ward) will not be paid anything in the form of material or money for your participation in the study. However, your participation will be highly appreciated.

Confidentiality

All the information collected will be treated with the utmost confidentiality and will be used for the intended purpose only. You (or your child/ward) will not be identified by name in any dissemination or publication resulting from this study.

Questions

If you have any questions concerning this study you can contact Alfred B Kayira, a graduate student of the University of Ghana on 0503268436 or 0554650799. Ethical clearance to do this study was obtained from the Ethical Review Committee of the Ghana Health Service. The chairman can be contacted on 0507041225 or 0243235225 or 0244712919 for any information concerning this study.

Consent form

I have been adequately informed of (or I have read and understood) the purpose, procedures, potential risks and benefits of this study. I have had the opportunity to ask

questions about it. Any questions that I have asked have been answered to my satisfaction. I know that I can refuse to participate (or have my child/ward participate) in this study without any loss of benefit to which I (or my child/ward) would have otherwise been entitled. I understand that any information collected will be treated confidentially. I freely agree to participate (or have my child/ward participate) in the study. After signing below I will receive a copy of the information sheet and this consent form.

Name of participant

Signature (participant/parent/ guardian):

Date:/...../..... (dd/mm/yyyy)

Name of parent of parent/ guardian (if applicable).....

Date:/...../..... (dd/mm/yyyy)

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

Name of study personnel:

Signature:

Date: (dd/mm/yyyy)

