EFFECTIVENESS OF RAPID DIAGNOSTIC TEST FOR MALARIA DIAGNOSIS IN CHILDREN UNDER 15 YEARS OF AGE OF NCHELENGE DISTRICT IN THE LUAPULA PROVINCE.

A dissertation submitted to the University of Zambia in Partial Fulfillment of the Master in Public Health

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DECLARATION

I hereby declare that to the best of my knowledge the work presented in this study, for the masters in Public Health has not been presented either wholly or in part for any other master's in Public Health Degree and is not currently submitted for any other degree.

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Having supervised and read this dissertation, we confirm that the work has been completed satisfactorily and befits the award of a master's degree in Public Health.

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STATEMENT

I hereby certify that this work presented for the Degree of Master in Public Health, is in all entirely the results of my own independent investigations. The various sources to which I am indebted are gratefully acknowledged in the text and in the bibliography

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APPROVAL

This dissertation of Israel Ndayambaje is approved in partial fulfillment of the requirement for the award of the degree in Master of Public Health by the University of Zambia.

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DEDICATION

This dissertation is dedicated to the memory of my Dear Dad and Mum, and my two Brothers for the physical, mental and spiritual support rendered to me

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ABSTRACT

Rapid detection of the malaria parasite and early treatment of infection still remain the most important goals of disease management. This study was conducted to evaluate the diagnostic performance of the Rapid Diagnostic Test (RDT) Paracheck Malaria *Pf* in a Zambian circumstance and its objective was to compare the Effectiveness of Rapid Diagnostic Test (RDT) to Microscopic examination in detecting malaria parasites.

Design: A cross sectional method was used in this study. The researcher collected information from sampled individuals using quantitative and qualitative methods. Three (3) Health centres in Nchelenge District were randomly selected. Thereafter, a systematic sampling procedure picking every 2nd child was used to select children (under 15 years of age), attending the diagnostic centres.

Main Outcomes: The figures for specificity, sensitivity, and predictive values were calculated in percentage using microscopy as the gold standard.

Results: The findings of this study demonstrated that the RDTs for detection of *malaria P. falciparum* was highly sensitive (96.1%) but less specific (53.8%) for the diagnosis of malaria plasmodium falciparum, with a positive predictive value (PPV) of 80.2% and a negative predictive value (NPV) of 87.6 %.

The sensitivity of the HRP-II RDTs correlated directly with parasite density and it was 100% at parasitemias of above 440/µl; however, the sensitivity dropped from overall 98 to 50% at parasitemias of less than 440/µl.

Conclusion: This study demonstrated that 96.1% of RDTs can diagnose malaria *P.f.* in an individual whether symptomatic or not. The Ministry of Health through the NMCC is intending to bring down the prevalence of malaria substantially by the year 2011; this target can be achieved if RDTs that have 96.1 % sensitivity are used as a major diagnostic tool in Zambia.

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ACRONYMS

B/S:	Blood Slides
CSO:	Central Statistical Office
DHMT:	District Health Management Team
FN:	False Negative
FP:	False Positive
HMIS:	Health Management Information System
HRP-II:	Histidine-Rich Protein type two
MACEPA:	Malaria Control and Evaluation Partnership in Africa
NMCC:	National Malaria Control Centre (NMCC
Pf:	*Plasmodium falciparum*
pLDH:	parasite Lactate Dehydrogenase
RBM:	Roll Back Malaria
TN:	True Negative
TP:	True Positive
RDTs:	Rapid Diagnostic Tests
WBCs:	White Blood Cells
WHO:	World Health Organisation

1. INTRODUCTION

1.1. BACKGROUND INFORMATION

Malaria is a serious illness caused by plasmodium parasites. There are four human Plasmodium namely *P. falciparum, Ovale, Vivax, and Malariae.* It is transmitted by Mosquito bite and less frequently by transfusion with unscreened blood and in some instances, transplacentally.

According to the world health organization (WHO), more than 40% of the world's population is exposed to the risk of malaria. Out of these, 270 million suffer from malaria, and, at least, over 500 million malaria cases per annum occur worldwide each year, resulting in more than one million deaths annually. About 89% of these deaths are estimated to occur in sub-Saharan Africa, mostly among children below the age of five years (WHO, 2002).

According to WHO (Global Health Report 2003) estimated 1,272,000 deaths occurred globally and Africa was leading with; 1,136,000 (89.3%), South-East Asia; 65,000 (5.1%), Americas; 1,000 (less than 1%), Western Pacific; 11,000 (less than 1%), Eastern Mediterranean; 59,000 (4.6%), Europe; 0 (0%).

Malaria epidemics became a regular feature of many parts of the world, including the highlands of Africa. The epidemics was associated with the warming of the climate, disruption of health services and large scale uncontrolled population movement due to social disruption and civil wars. Proliferation of drug resistance is closely related to

massive population movements coincided with inadequate health services, improper use of antimalarial drugs, limited resources and operational difficulties in implementing malaria control activities (Kondranchine, 1997.)

Malaria continues to be one of the main public health problems in the world, especially in a majority of African countries. It particularly affects young children, young adults engaged in economic development activities, pregnant women and international itinerant groups, moving into malaria endemic areas. The economic impact of malaria is felt by various social groups of society (particularly in the poorest countries of the world) and among populations living under the most difficult conditions (WHO, 2002). To overcome malaria challenges, there is a need for concerted efforts by health services, private sector, local and international communities.

A prompt and accurate diagnosis is the key to effective disease management. The two diagnostic approaches currently used in developing countries most often, are clinical diagnosis and microscopy diagnosis; however, these do not allow a satisfactory diagnosis of malaria. Of the two, Clinical diagnosis is the most widely used approach; but, the sings and symptoms of malaria are very non-specific and overlap those of other febrile illnesses (World Health Organization, 1999). A diagnosis of malaria based on clinical grounds alone is therefore unreliable, and, when possible, should be confirmed by laboratory tests.

Microscopic examination of thick blood film is currently the "standard" method for malaria diagnosis. It is relatively a simple method and has low direct cost, it is sensitive to a threshold of 5 to 50 parasites/μl depending on technical factors such as the expertise of the microscopist (Moody, 2002). Microscopic examination is also able to characterize the infecting species and their relative densities. However, its reliability is dependent on skills of laboratory microscopist, particularly at low levels of parasitaemia and in the interpretation of mixed infection. Also it requires Laboratory facilities, enough light/electricity, and water, and room, space including considerable technical expertise for optimal blood film preparation, examination, and interpretation.

Furthermore, currently Zambia is facing human resource crisis and health systems are working on 50% man power, besides the majority of the clinics do not have Laboratory facilities (MoH, 2004), hence there is a delay in carrying out microscopic examination and arriving at malaria diagnosis. Recently, rapid antigen detection methods have been developed for situations in which reliable microscopy may not be available. These tests are based on the detection of antigen(s) released from parasitized red blood cells (Moody, 2002). In the case of *Plasmodium falciparum*, these new methods are based on detection of *P. falciparum* histidine-rich protein II (HRP-II) (*Para*Sight F, Australia or *Plasmodium*-specific lactate dehydrogenase (pLDH)). Species-specific pLDH isoforms have been used to develop a test for *Plasmodium vivax* (OptiMAL). (Moody, et al, 2000).

Paracheck is one of the rapid tests that detect the presence of *Plasmodium falciparum* (*P.f*) from blood samples; it utilises the principle of immunochromatography in blood. It is an indirect method of detecting P.f as it detects the HRP-II antigen secreted by the trophozoite and gametocyte the moment that they invade the red blood cells. As the test

sample flows through the membrane assembly of the dipstick after placing into the clearing buffer tube, the colored anti P.f HRP-II antiseracolloidal gold conjugate (monoclonal) complexes the P.f HRP-II in the lysed sample. This complex moves further on the membrane to the test region where it is immobilised by the anti P.f.HRP-2(monoclonal) antisera coated on the membrane leading to formation of a pink colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any, move further on the membrane and are subsequently immobilised by anti rabbit anti bodies coated on the membrane in the control region, forming a pink band. This control band serves to validate the test performance. (Cooke, et al 1999)

Malaria is a major public health problem in Zambia. It is the leading cause of morbidity and mortality, accounting for 45% of all hospitalizations and outpatient attendances and 50% of cases among children under-five years of age (HMIS 2004). The National Malaria Control Centre (NMCC) estimates that malaria is responsible for nearly 4.3 million clinical cases and over 50,000 deaths per year, including up to 20% of maternal mortality and malaria incidence in 2004 was 383 per 1,000 population. The prevalence rate of Malaria in Zambia as estimated by National Malaria Indicator Surveys has been at 21% and 16% in 2006 and 2008 respectively (MoH, 2008).

The vision of the Zambian Government has been "To provide Zambians with equity of access to cost effective health care as close to the family as possible." In the case of malaria diagnosis, it is addressed through the provision of laboratory services as close to the family as possible.

Nchelenge District was selected for the purpose of this study mainly because malaria prevalence in Zambia is highest (37,5%) in Luapula province (MoH National Malaria Indicator Survey, 2006). Nchelenge District is located in the northern part of Luapula Province 250 km away from Mansa, the Provincial Capital. The total area of Nchelenge is 4000 square kilometres of which 2391 km sq (60%) is land, 400 km sq (10%) is swamps and 1231 km sq (30%) is water. Nchelenge District shares borders with Chienge District in the North, Kawambwa District in the South-East, and The Democratic Republic of the Congo in the West with Lake Mweru and Luapula River forming natural boundaries. The main natural features of the District include the Luapula Valley, Lake Mweru, Luapula River and dambos. There are significant Islands like Isokwe, Kanakashi, Kilwa and Chisenga. The total population of Nchelenge District is about 1,129,830 (CSO, 2000).

1.2. STATEMENT OF THE PROBLEM

Malaria is an endemic disease, one of the biggest killers in sub-Saharan Africa (WHO, 2004). It is documented to be the most common cause of morbidity and mortality in both rural and urban areas and It features among the first five diseases causing morbidity and mortality in Zambia. Malaria is a major contributing factor to maternal mortality of 449 per 100,000 live births (of which 20 % is from malaria) and infant mortality of 70 per 1,000 live births of which 40% are due to Malaria (MoH, 2008). Malaria adds to the already existing anaemia during pregnancy, leading to low birth weight and premature babies and infant and maternal deaths (NMCC, 1999)

Nchelenge district was found to have the highest malaria prevalence in Zambia that is 37.5% (MoH, 2006). In Zambia, the problem of malaria was estimated at 388/1000 people in 2004, (MoH 2006). Malaria influences economic status of individuals from house holds up to national level. The ever-shrinking economy has brought in its wake, drastic cuts in expenditure on social services such as health, education and agriculture. The cut in national budget means shortage of essential drugs, manpower and other preventive interventions (like ITN and vector control programme). On the other hand, the high rate of poverty has brought a rapid rise in housing, which allows easy entry of mosquitoes. Individual household members cannot afford to buy ITNs or pay for health services once they are sick. In addition, laboratory technicians do not attend to the majority of cases because they are few and they are work overloaded (MoH, 2006).

The high rate of self-administering of malaria drugs and clinically diagnosing of malaria is a source of concern that is tripled by malaria misdiagnosis, which is estimated at 34 % in Zambia (NMCC NSP 2006-2010).

All these circumstances increase malaria resistance and make it difficult to control the disease. Complications due to self-administration of drugs, malaria resistance and malaria mortality rate result unless people have access to laboratory test for malaria parasites detection. Diagnostic Laboratory services are accessed from well-equipped health institutions but these can only be accessible at hospitals and selected few clinics (diagnostic centres) under DHMT. Therefore, the Serological test using RDT dipstick has been found to be useful and demand less technicality to come up with results (Makler and Palmer, 1998).

In 2006, MoH through National Malaria Control Centre (NMCC) carried out Malaria Indicator Survey (MIS) Supported by MACEPA where Paracheck HRP-II dipsticks were used but the majority of respondents were refusing to be tested and out of those tested very few came out positive with the RDTs (The final report doesn't separate Microscopic and RDT parasaetemia results). However, these serological tests are being introduced in all the Districts of Zambia and need to be tested locally. It is therefore important to assess, analyse and compare RDT with microscopy test and determine the effectiveness of RDTs and create environment of encouraging health care planners, deliverers and the community to use the easiest, simplest and most effective way of detecting malaria parasites.

1.3. LITERATURE REVIEW

Malaria is an illness caused in man by infection with protozoa of the genus plasmodium. There are four human Plasmodium namely P. *falciparum, Ovale, Vivax, and Malariae.* In Zambia about 90-95% of Malaria cases are due to the malignant P.*f.* (NMCC 1999)

Malaria is one of the world's most common and serious tropical diseases that causes at least one million deaths every year (WHO, Global Health Reporting, 2000). The majority of which occur in the most resource-poor countries and more than half of the world's population is at risk of acquiring malaria. The proportion of those affected with malaria increases each year, because of deteriorating health systems, resistant insecticides and drugs, climate change, natural disasters and armed conflict.

The State of the Pandemic as stated by the WHO (2003) is that more than 300 million acute cases of malaria occurred in 2002 worldwide, resulting in more than one million deaths annually. More than 80% of these deaths are estimated to occur in sub-Saharan Africa, mostly among children under five years old. Malaria accounted for one in 10 deaths among children in developing countries in 2002.

Recent estimates of the global burden of malaria are even higher, with one study estimating that 515 million cases of clinical malaria occurred in 2002; approximately 60% of all cases of malaria occurred among the poorest 20% of the world's population. Overall, malaria accounts for 10% of Africa's disease burden; it is estimated that malaria costs the continent more than $12 billion annually. Although Africa is hardest hit, it is estimated that more than one-third of clinical malaria cases occur in Asia and 3% occur in the Americas (WHO 2005). The burden of malaria has been increasing, The WHO and Roll Back Malaria (2005) has estimated that the mortality caused by malaria increased up to more than 100 million deaths each year and that 3.2 billion people around the world are at risk of malaria. The RBM initiative is a partnership between malaria affected countries in Africa and WHO, its major objective is to harness the political will and help to create the necessary economic and social conditions necessary to develop appropriate tools that can be used by all communities affected by malaria, especially the poorest. The long term-goal for RBM is to halve the malaria mortality for Africa's people by 2010. This goal is in accord with the 6^{th} Millennium Development Goal (MDG) of combating HIV/AIDS, Malaria and other major diseases.

Zambia being in the sub-Saharan Africa is also heavily affected. Malaria is a very serious Public Health problem accounting for 3 million clinical cases and 50,000 deaths every year. The problem of malaria has been increasing since the 1970's to date. In 1976 malaria incidence was at 121.5 per 1,000 people, in 1999 it was at 308 per 1,000 people and in 2003 Malaria incidence was at 482/1,000. In 1976, out of the total health care attendance, malaria Prevalence stood at 10.2%. This doubled to 21% in 1992 (NMCC, 1999). However, the Government of Zambia has recently recorded a decrease in malaria prevalence to 16% (MoH, 2008) and this is attributed to massive malaria interventions such as In-Door Residual Spaying(IRS), distribution of Insecticide Treated Mosquito Nets (ITN), Intermittent Presumptive Treatment (IPT) coupled with appropriate diagnosis using RDTs .

Malaria predominantly affects children; the infant mortality rate in 2006 was at 95/1,000 (40% due to malaria) while the maternal mortality rate was at 729/100,000 (20% due to malaria). Also, malaria misdiagnosis is high in Zambia. Currently it is at about 34 %. (MoH, 2006).

Zambia National Malaria Indicator Survey reported that Malaria is higher in rural areas than in urban areas (28.9% Versus 4.9% respectively); the highest levels of malaria prevalence was reported in Luapula Province at 37.5% and the area with the lowest rate was Lusaka at 0.7% (MoH, 2006).

Microscopy is a valuable technique when done properly, but unreliable when poorly performed. As the detection limit of microscopy is $\pm$ 20 parasites/μl of blood or even

more realistically at 50/μl, a substantial number of patients with low numbers of parasites may be missed using this technique (World Health Organization, 1999).

Malaria presents a diagnostic challenge to laboratories in most developed countries due to technicality required, and endemic malaria, population movements, and, travelers, all contribute to presenting the laboratory with diagnostic problems for which it may have little expertise. Furthermore, microscopy is time consuming and labour intensive. (Moody, 2002).

Malaria rapid diagnostic devices have been developed with the hope that they would offer accurate, reliable, rapid, cheap and easily available alternatives to traditional methods of malaria diagnosis. The results from early malaria rapid diagnostic studies have been quite promising, especially, for detecting *Plasmodium falciparum* at densities of more than 100 parasites/μl compared to about 20 parasites/μl in Microscopic examination. Despite the introduction of these devices over a decade ago, only few target antigens have been introduced. Of greater concern, these devices have shown limitations in sensitivity, inability to differentiate species and lack of robustness under field conditions in the tropics. Recent trials have revealed wide variability in sensitivity both within and between products (Murry et al, 2003). The limitation of Laboratory services in Zambia is quite significant with 16% of laboratory facilities available countrywide and only 34% of the Zambian population access laboratory Diagnostic in serviced Health Centers (MoH 2006).

The appropriateness of microscopy versus antigen-detecting rapid diagnostic tests depends on a number of factors including parasite density, availability of skilled

personnel and resources, and the capacity for maintaining quality assurance of microscopy and RDT, and the need for quantitative assessment of parasite density (Hanscheid, 1999). RDTs that are kept in good condition, can achieve sensitivity similar to that commonly achieved by microscopy. Sensitivity can vary between products. Recommended sensitivity is 95% at 100 parasites /µl for P. falciparum (WHO, 2003).

Rapid diagnostic tests, in simple kit form, can provide results based on fingerprick or venous blood within minutes. They can be used by village health workers after as little as an hour of training (Makler and Palmer, 1998). Rapid Diagnostic Test (RDT) is a qualitative (presence or absence of Plasmodium) and not a quantitative measure; It cannot quantify the parasitaemia. The result is obtained within 15 minutes (as per manufacturer instruction) using a very simple technique. This test has a very high sensitivity (95 percent), so it is as reliable as a skilled microscopist. Its greatest disadvantage is that it remains positive for seven to 14 days after treatment. There is, thus, no point in re-doing the test during that period (WHO 2004).

In an unpublished report, WHO did a study comparing some RDT on the market, and found that HRP II-detecting tests are likely to have greater sensitivity than pLDH; Aldolase-detecting tests are currently used for detection of *P. falciparum* infection in most environments (WHO,2005). In a study conducted by Ngamngonkiri (1999), the author compared microscopy test with Paracheck- *Pf*"dipsticks and showed that "there were five false positive results with the Paracheck-Pf dipsticks": four cases had a recent history (with a range of one week to three months) of *P. falciparum* infection; three were malaria smear negative, and, one was diagnosed as P. *vivax* by microscopy. The fifth case had a history of *P. malariae* infection four months previously. One false negative test was

found on microscopy to have a mixed infection with both *P. falciparum* (1 trophozoite and 1 gametocyte per 500 WBC) and *P. vivax* (2 trophozoites per 500 WBC).

The potentiality of RDT during transportation and storage is an issue that has not yet been ascertained. WHO (2005) suggests that all batches of RDT should be tested after procurement and monitored throughout shelf-life. Evidence of good manufacturing practice and good field experience should be considered during procurement. Standard specifications for procurement, also, should be followed. The WHO recommends that, temperatures above 30°C are considered inappropriate for storing RDTs. Temperatures above 30°C can affect overall performance of the RDTs, temperature-monitoring needs to take place in all clinics and environments. Air-conditioning or similar cooling equipment should be considered in those clinics that exceed the WHO recommended threshold (or manufacturers instructions) (WHO, 2003).

1.4. JUSTIFICATION

The Zambian government's vision of "equity of access to cost-effective health care as close the family as possible" implies that, rural and urban Zambian people should have access to malaria detection of parasites services before treatment as early as possible. This will help avoid, misuse of drugs, or, self administering of malarial drugs, which may lead to malaria resistance or complications (which may increase malaria mortality). Better Services, including detection of malaria parasites within the shortest period of

time, increases utilisation of health services and decreases the proportion of malaria resistance and mortality.

Microscopic examination of blood smears is widely recommended and used as a routine method for detection of malaria parasite, and, remains the gold standard for malaria diagnosis (Cooke, 1999). But, microscopic examination is laborious and requires considerable expertise for its interpretation, particularly at low levels of parasitaemia. In addition, in patients with *plasmodium falciparum* malaria, sometimes the parasites can be sequestered and are not present in peripheral blood. Thus, a *P. falciparum* infection could be missed due to absence of the parasite in a blood film. Currently Zambia is trying to refrain from clinical case definition as Malaria diagnosis resorting to more practical methods of diagnosis that are easy and quick to give evidence of the presence of the malaria parasite under microscopy or rapid method of diagnosis (RDT), Besides these, majority of malaria cases occur in rural areas where there is little or no access to laboratory services; in many areas, microscopy is not available and shortage of health personnel remains a constraint to health care delivery (Schallig and Schnnel, 2003)). WHO acknowledges that shortages of skilled personnel impede malaria management and control more than the lack of financial resources, and, the management of malaria, which includes the use of parasite-based diagnosis like RDT, contributes to improved malaria management and potential savings of health resources (WHO, 1999). RDTs suit various circumstances because they can be easily used by Community Health Workers (CHWs), giving opportunity of malaria parasites detection to all Zambians especially where Microscopic services are not available.

The study will compare microscopic examination of blood film with newly developed dipstick antigen capture which detects Histidine Rich Protein (HRP-II) antigen of malaria parasites in order to identify strengths and weaknesses of RDT. Findings learnt from the study will be used to recommend RDT method of diagnosis to improve malaria management.

2. OBJECTIVES

General objective: To compare the Effectiveness of Rapid Diagnostic Test (RDT) to Microscopic examination in detecting malaria parasites.

Specific objectives:

1. To determine the Sensitivity and Specificity of RDTs.
2. To determine the Predictive Value of RDTs.
3. To establish factors affecting the serological and microscopic examination.
4. To describe the serological findings in relation to microscopic examination
5. To make recommendations for the use of the serological test

3. HYPOTHESES

Ho: The Sensitivity of RDTs is < or = 90 % and Specificity of RDTs is < or = 80%.

Ha: The Sensitivity of RDTs is > 90 % and specificity of RDTs is > 80 %.

4. VARIABLES

Independent Variables:

1 Demographic data

2 Rapid diagnostic test

3 Microscopic testing

4 Factors affecting end results of tests:

   a. Previous use of Malarial drug

   b. Duration from the onset of disease

   c. Presenting signs and symptoms

   d. Type of plasmodium

5 Any history of fever/malaria before the onset of disease.

6 Parasites present (number, viability)

Dependent Variables:

Detection of malaria parasites

Definitions:

(1) *Serological test*: Blood testing using Rapid Diagnostic Tests (RDTs) to identify malaria antibody in the blood serum.

(2) *Microscopic test*: use microscopy to identify malaria parasites

(3) *Factor*: an influence that tends to produce results.

(4) *Effectiveness*: The ability of RDTs to desirably detect Malaria parasites in actual usage.

(5) *Specificity*: It is the probability that a RDT test will produce a true negative result When used on a noninfected population (as determined by a microscopy as a reference or "gold standard").

(6) *Sensitivity*: It is the probability that a RDT test will produce a true positive result when used on an infected population (as compared to a reference or "gold standard").

(7) *Positive Predictive Value*: It is the probability that a person is infected when a RDT positive test result is observed.

(8) *Negative Predictive value*: It is the probability that a person is not infected when a RDT negative test result is observed.

5. METHODOLOGY

5.1. Research design

A cross sectional method was used including quantitative techniques of Data analysis. This research spells out the basic strategies that were adopted to develop information that was accurate and interpretable.

5.2. Study setting

The study was conducted in Luapula Province, Nchelenge District that has got a population of 1,129,830 (CSO 2000 National Census). It is served by one hospital and 6 Diagnostic Health Centres that have Laboratory facilities.

Nchelenge District was selected as study area because of its highest malaria prevalence in Zambia that is 37.5% (MoH, 2006). Nchelenge has got natural features that may be contributing to its high malaria prevalence such as Surface waters which accounts for 30% of the total Nchelenge Surface (Lake Mweru, Luapula River), and Swamps and Damps (2006 Nchelenge District strategic Plan).

5. 3. Data collection technique.

The data was collected from sampled respondents. This study used quantitative and qualitative methods.

Peripheral blood smear examination for malarial parasites were collected at the same time for both blood slide and RDT for each child under 15 years of age who volunteered or/and whose parents provided ascent to participate in the study. Each respondent was asked about signs and symptoms of malaria and medication taken during the prior 3

weeks. Thick and thin blood smears were made by using the edge of the slide and another with the corner of slide. Slides were stained with Giemsa stain 10% and examined under light microscope. Thick smears were examined for presence of parasite, while thin smears were evaluated for species of parasite. At least 100 high power fields were scanned in each slide. Two (2) laboratory technicians (who have been dealing with the blood slides for at least the past 4 months prior to this study) examined each Blood slide for all the samples independently and were blinded of the RDT results, and a 3rd was used to ascertain results that were found conflicting.

Results of thick and thin smears were recorded on the questionnaire whether they were positive or negative for malaria for each child.

For the same finger prick, (Paracheck HPR-II) *Pf* strips testing the presence of HRP II antigen were used in the study. The Paracheck were brought to room temperature and finger prick blood delivered to its designated area of the dipstick. Three (3) drops of clearing buffer was dropped on to the sample pad just below the arrows on the dipstick as per manufacturer instructions. After 15 minutes, the readings were noted. The test was considered valid if only one control pink color line was visible on the dipstick and as positive for falciparum, if the second HRP-II line of distinct color bands appeared. The results obtained from the Microscopy and RDTs (Paracheck strips) where then compared for specificity and sensitivity.

5.4. Data collection tools

The instruments used in this study were structured questionnaires, paracheck dipsticks for serological test, lancets, and slides for thick and thin blood slide and microscope.

5.5. Sampling

5.5.1 Study population

Study population were children under 15 years of age attending laboratory centres. For their participation, written consent was obtained from their parents.

5.5.2 Sample selection

Three (3) Health centres in Nchelenge District were randomly selected and Saint Paul's OPD was captured along side the three health centres. Thereafter, a systematic sampling procedure picking every 2^{nd} child was used to select children (under 15 years of age), attending the diagnostic centres and two (2) Health centres had equal numbers of sample and the Hospital OPD sample size doubled other centre's number. Only those meeting the study criteria were selected to be in the study.

5.5.3. Sample size

The sample size was children under 15 years of age and to arrive at the sample size the following standard formula was used:

$N= Z^2 pq /d^2$

$Nf= n/n+1/N$

$\mathbf{Z}^2$ being 95% confidence level

d being error level

Pq being the maximum outcome assumed

nf being sample size with target population in mind

N being target population

The investigator expected 80% of clients who have positive blood slides would have RDTs positive results (This group represented the Sensitivity sample). Therefore, the expected proportion is 20% (100-80)

$n = (1.96)^2 \times 20 \times 80/5^2$

$=245$

Considering 90% response rate, the sample size calculated at:

245:0.90= 272 and 280 clients were considered to be the sample size.

The investigator expected 90% of clients have negative blood slide would have RDT negative results (This group represent the Specificity sample). Therefore the expected proportion was 10% (100-90)

$n = (1.96)^2 \times 10 \times 90/5^2$

$=138$

Considering 90% response rate, the sample size was:

138:0.90= 153 and 155 clients were considered to be the sample size.

Inclusion Criteria: Included Child below 15 years old, whose parents/guardians provided ascent for finger prick, attending Laboratory services for malaria test after being clinically screened for suspected malaria and had not received Malaria treatment in the previous two weeks.

Exclusion criteria: Anyone aged 15 years and above, and all those who have received malaria treatment in the last two weeks were excluded. Those who did not give consent were excluded from the study; participation was purely on voluntary basis.

5.6. Validity

External validity: All samples (for RDTS and microscopy) were collected at the same time as the malaria level fluctuates rapidly. Also interviews were held in the same manner to all caretakers.

Internal validity; this refered to instrument used and man power. The interview schedules were tested by a pilot study. The investigator ensured a good condition of paracheck sticks (the cool chain for RDTs was maintained) and slides. The Researcher used 2 laboratory technicians to examine blood slides for all the samples and a 3rd to ascertain those whose results were found conflicting.

5.7. Reliability

The respondents were assured of confidentiality to ensure that they awere at ease at time of interviews. Also all research assistants were trained to ensure a good understanding of the instruments and how they are used. They were given knowledge and any information about Paracheck dipstick used to solve some inconsistencies.

The sticks, slides and the questionnaires carried the same respondent number. The research assistant read and recorded the results immediately after testing.

Deviation from protocol; the principal investigator followed up the research assistants to take care of any anticipated enrolment of any study subject not meeting the inclusion and exclusion criteria. Documentation of any issues/observations encountered during the study were completed on the questionnaire

5.8. Pilot study

A pilot study was conducted in two randomly selected Lusaka District Health centres, to pre-test and assess the validity of data collection tools and methodology. 10% of the total sample was used as respondents for pilot study. Minor adjustment to the questionnaires, the process of data collection and the use of RDTs were done following the pilot study.

5.9. Ethical consideration

In order to protect the rights of the clients, ethical clearance was requested from the UNZA Research Ethics Committee. Also the researcher obtained a written permission from the relevant authority; this was from the Head of Department of Community Medicine, Luapula Provincial Health Director, Saint Paul's Mission Hospital Director, Nchelenge District Director of Health and sampled diagnostic Health Centre In-charges. Permission from Respondents' caretaker was also obtained and they were clearly informed of their rights. The purpose and nature of the study was explained to clients as well as how the findings are to be utilised.

Clients were assured of confidentiality and anonymity by using serial numbers on their questionnaires instead of names, and their guardians were asked for their willingness to participate in the study (consent forms were given to participants- see Annex I for a copy).

5.10. Plan for data collection

Training of Two research assistants was done in the last 2 weeks of March, 2008, to equip them with knowledge and skills needed to use RDT to test malaria among Children. (See page 18 for Ghant Chart).

Data collection was done during the whole month of April and 2 weeks of May, 2008. The Principal Investigator together with the Research Assistants collected data and blood samples (for RDTs and B/S for mps) from under 15 years children attending selected Diagnostic centres. The interviews were conducted using structured interview schedules and these interviews were followed strictly to avoid cross-examining respondents.

5.11. Adverse reaction

No side effects or adverse reactions were expected. However, minor effects were experienced in this study. The experienced risks of a finger prick were pain, slight discomfort and/or a small bruise at the site of puncture. No infection or swelling to the surrounding area was recorded. All normal technical precautions were taken to these risks and the above mentioned risks were very few. These minor incidences were recorded and managed appropriately by a clinician.

5.12. LIMITATION OF THE STUDY

The main limitation of this study was that the study was conducted in health facilities and this posed a challenge in selection of respondents who are truly negative for the specificity group and this could have lead to low specificity found in this study.

6. PLAN FOR DATA PROCESSING AND ANALYSIS

Data was checked for accuracy, completeness, and consistency in responses.

Data was categorised, coded, entered and analysed using EP Info V6. The study findings are presented in table form, with their statements to summarise results in meaningful way and easy interpretation of findings.

Data collected were quantified in numerical values and percentages for easy manipulation using statistical procedures for the purpose of describing phenomenon or assessing the magnitude and reliability of relationship among them. For statistical analysis, RDTs results were classified as true positive, true negative, false positive or false negative for each test under evaluation compared with the reference standard. Confidence intervals (CI) were calculated at 95% for sensitivity and specificity using Baye's theorem.

TABLE 1: Standard table used for easy computation of sensitivity, specificity, and negative and positive predictive values

New Test Results (RDT)	**Reference Test Results (microscopy)**	
	Positive	Negative
Positive	***True Positive (TP)***	***False Positive (FP)***
Negative	***False Negative (FN)***	***True Negative (TN)***

Sensitivity, specificity, positive and negative predictive values were calculated as follow;

i. ***Sensitivity: TP/ TP + FN,***

ii. ***Specificity: TN/ TN + FP,***

iii. ***Negative Predictive Value: TN/ TN + FN, and,***

iv. ***Positive Predictive value: TP/ TP + FP.***

Also quantification of parasites was compared with specificity and sensitivity of the RDTs. Parasites in thick blood films were counted against 200 white blood cells or 500 in cases of low parasiteamia. The parasite density was estimated assuming 8,000 white blood cells/µl of blood; the formula used was as follow:

Number of Parasites/µl blood = (parasites/200WBCs) × 8,000 WBC or

parasites/200 WBC × 40

The results were presented and discussed in relation to other findings from similar studies conducted elsewhere.

7. DISSEMINATION AND UTILISATION OF RESULTS

Findings of the research project will be disseminated to the National malaria Control Centre, the Funders of the research, Research Ethics Committee, St Paul's Mission Hospital and Nchelenge District Health Management Team (NDHMT). The results will be used to improve malaria diagnosis in the country and promoting the use of Rapid Test Diagnosis in Zambia.

8. DATA ANALYSIS

8.1 INTRODUCTION

This study sought to test the accuracy of the Paracheck *Pf* bland of HRP-II RDTs for *P. falcifarum*, and finger prick blood samples were collected from respondents and tested using the RDTs and microscopy. All respondents were children under 15 years of age attending the clinic with signs and symptoms of suspected malaria. Interviews were conducted asking questions to Mothers or Guardian who brought these children to Clinics or Hospital. A total number of 437 respondents (Mothers or Guardians) were interviewed and general signs and symptoms of malaria and their children's past health status were recorded. Out of a total sample size of 437, 48 samples did not meet the criteria for Laboratory investigation leaving a sample size of 389 only for data analysis of this study. This chapter presents the analysis of these findings in table form for easy interpretation and discussion.

9.2. DEMOGRAPHIC CHARACTERISTICS

TABLE 2. Sex and Age Distributions of respondents

	FREQUENCY	PERCENTAGE
SEX		
Female	217	55.8
Males	172	45.1
TOTAL	**389**	**100**
AGE		
0-4 Years	191	49.1
4.1 – 9 Years	98	25.2
9.1 – 14 Years	100	25.7
TOTAL	**389**	**100**

Table 2 shows that most of the respondents were female 217 (55.8%), and the majority of respondents were falling in the age group of Zero to 4 years 191 (49.1 %), followed by the group aged 9.1 to 14 years of age 100 (25.7 %) and those aged 4.1 to 9 years of age 98 (25.2 %).

9.3 HEALTH STATUS OF RESPONDENTS

TABLE 3: Signs and Symptoms presentations

SIGNS & SYMPTOMS	FREQUENCY	PERCENTAGE
Yes	342	88
No	47	12
TOTAL	**389**	**100**
Fever	316	81.3
Others	26	6.7
No signs & symptoms	47	12
TOTAL	**389**	**100**

The table 3 shows that the majority of respondents 342 (88%) had sings and symptoms suggesting malaria and 47 (12%) had no signs. Out of the total respondents who presented with general signs and symptoms of malaria 316 (81.3) had a history of fever.

TABLE 4: History of any other disease that occurred before the current illness

PREVIOUS HISTRY OF DISEASE	FREQUENCY	PERCENTAGE
NO	364	93.6
YES	25	6.4
TOTAL	389	100

Table 4 shows that 25 (6.4 %) had a history of previous illnesses.

TABLE 5: Rapid Diagnostic Tests findings

RDTS	FREQUENCY	PERCENTAGE
Positive	308	79.2
Negative	81	20.8
TOTAL	389	100

Table 5 shows that 308 (79.2%) were RDTs Positive and 81 (20.8%) were RDT negative.

TABLE 6: RDT and SEX

	RDTS				TOTAL	
SEX	Positive	%	Negative	%		%
FEMALE	179	58.1	36	44.5	215	55.3
MALE	129	41.9	45	55.5	174	44.7
TOTAL	308	79.2	81	20.8	389	100

Table 6 shows that the total number of children who were positive were 308 of which 179 (58.1) were females and 129 (55.5%) were males.

TABLE 7: Respondents' RDT results and their history of Fever

	RDT					
History of Fever within the previous 2 weeks	Positive		Negative		Total	
	Freq	%	Freq	%	freq	%
Yes	265	86	51	63	316	79
No	43	14	30	37	73	21
TOTAL	308	79	81	21	389	100

Table 7 shows that out of the total respondents who had fever 316 (79 %), 269 (86 %) were RDT positive and 51 (63 %) were RDT negative, and within those who had no fever 73 (21 %), 43 (14 %) were RDT positive and 30 (37 %) had RDT negative. On the whole, the RDTs were successful in identifying the malaria antibodies in majority of cases even among those who did not give any history of fever.

TABLE 8: RDT and history of any previous disease.

History of previous illness	RDT				TOTAL	
	Positive		Negative			
	Freq	%	Freq	%		%
Yes	11	44	14	56	25	6.4
No	297	81.5	67	18.5	364	93.6
TOTAL	308	79.2	81	20.8	389	100

Table 8 shows that out of the total respondents 25 (6.4 %) who had any previous history of illness, 11 (44 %) had RDTs positive.

TABLE 9: Blood slide findings

MPS	FREQUENCY	PERCENTAGE
Positive	257	66
Negative	132	34
TOTAL	389	100

Table 9 shows that out of the total respondents 389, 257 (66 %) were blood slide malaria positive and 132 (34 %) were blood slide malaria negative, against 308 (79.2%) RDT positive and 81 (20.8%) RDT negative (Table 5), resulting in RDTs becoming more sensitive than blood slides.

TABLE 10: Parasite Density

PARASITE DENSITY	FREQUENCY	PERCENTAGE
40 to 200/ µl	169	65.8
240 to 440/ µl	20	7.8
480 to 112680/µl	68	26.4
TOTAL	257	100

Table 10 shows that the majority of respondents 169 (65.8%) had malaria parasite density ranging from 40 to 200 parasites per microliter.

TABLE 11: Comparing RDTs to microscopic results

	MPS				***TOTAL***	
RDTs	Positive		Negative			
	Freq	%	Freq	%	Freq	%
Positive	247	96.1	61	46.2	308	79.2
Negative	10	3.9	71	53.8	81	20.8
TOTAL	257	66	132	34	389	100

Table 11 shows that out of the total respondents who had Malaria blood slide positive 257 (66%), 247 (96.1%) had their RDTs positive and out of those who had malaria blood slide negative, 71 (53.8 %) had RDTs negative.

Sensitivity: TP/TP+FN= 247/257= 0.9610 (96.1%). 95% CI 93.7-98.5

Specificity: TN/TN+FP= 71/132= 0.5378 (53.8%). 95% CI 45.3-62.3

Negative predictive value: TN/TN+FN=71/81= 0.8765 (87.6%)

Positive predictive value: TP/TP+FP = 247/308=0.8019 (80.2%).

Table 11 shows that, RDTs are as sensitive as microscopy (sensitivity of 96.1%) meaning they are 96.1% capable of producing true positive results when used on an infected population but less specific (specificity of 53.8 %) or less effective as they have shown to produce about 53.8% of true negative when used on uninfected population as compared to microscopy as the "gold standard" in this study. Regarding the positive and negative predictive values, this study shows a high probability that persons are infected when RDT positive result is observed or they are not infected when an RDT negative is observed at 80.2% and 87.6% respectively.

Table 12: The relationship of Days of clients' History of fever to microscopy and RDT results

Days	mps +ve						mps -ve						**TOTALS**	
	RDT +ve		RDT -ve		**TOTAL**		RDT +ve		RDT - ve		**TOTAL**			
	Freq	%	freq	%	freq	%	freq	%	freq	%	freq	%	FREQ	%
No fever History	33	13.3	3	30	**36**	**14**	10	16.4	27	38	**37**	**28**	***73***	***18.7***
1-2 days	72	29.1	3	30	**75**	**29.2**	6	9.8	11	15.5	**17**	**12.8**	***92***	***23.6***
3-4 days	114	46.1	4	40	**118**	**46**	32	52.4	19	26.5	**51**	**38.6**	***169***	***43.5***
5-6 days	16	6.5	0	0	**16**	**6.2**	13	21.3	6	8.5	**19**	**14.3**	***35***	***8.9***
7-8 days	9	3.6	0	0	**9**	**3.5**	0	0	5	7	**5**	**3.8**	***14***	***3.6***
10-11 days	1	0.4	0	0	**1**	**0.4**	0	0	1	1.5	**1**	**0.7**	***2***	***0.5***
12-13 days	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	***0***	***0***
14 days	2	0.8	0	0	**2**	**0.8**	0	0	2	2.8	**2**	**1.5**	***4***	***1***
21-22 days	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	***0***	***0***
	247	100	10	100	**257**	**100**	61	100	71	100	**132**	**100**	***389***	***100***

Table 11 shows that out of total respondents 389, 383 (98.5%) had a history of fever of up to 8 days of which the majority 254 were RDT positive; and out of the total of respondents with RDT positive and slide negative 61, 32 (52.4%) had a history of fever for 3 to 4 days and 10 (16.4%) had no history of fever. This table shows that history of fever correlate more with the positivity of the RDT than blood slide and there were more samples that became RDT positive with negative blood slide than blood slide positive with RDT negative.

9. DISCUSSION OF RESULTS

The advent of easy, rapid, and accurate tests for the detection of Plasmodial infection is highly desirable. This study aimed at testing the paracheck brand of HRP-II Rapid Diagnostic tests for *Plasmodium falciparum*, they were compared to microscopy, with samples from symptomatic and non symptomatic suspected malaria clients. This study was done in clinical set up (a hospital OPD and two Health Centers) of Luapula Province, Nchelenge District (Zambia). This chapter presents the discussion of the main findings from the study and identified a number of merits and demerits of using the RDTs to detect malaria.

9.1. Demographic characteristics.

The majority of respondents were females 217 (55.8%). These findings correlate with ZDHS which states that females (51.7%) are more in the population than male (ZDHS 2001-2002). 191 (49.1 %) of the total respondents were young children of 0 to 4 years of age, this is in agreement with the World Health Report where it is stated that "Many children experience initial malaria infection during their first two years of life, as they have not yet developed sufficient immunity, making these early years particularly dangerous" (WHO,2006).

9.2. Characteristics of respondents and their health status presentation

The majority of respondents 342 (88 %) had signs and symptoms of malaria, and 316 (81.3%) had fever and only 25 (6.4 %) had signs of any illness prior to the onset of the current disease. This is mainly because the study was conducted in a clinical set up and

the investigator was targeting those that were presenting with clinical signs and symptoms assumed to be due to malaria.

9.3. Laboratory results

A total of 437 respondents were drawn from clients who were attending the laboratory facilities, 48 slides were removed from the total sample because they did not meet the criteria for laboratory investigations leaving the study with 389 samples. Among the samples, positive blood slides for malaria (use of microscopy) were found in 257 (66 %) children with the remaining slides, 132 (34%) found negative (Table 9). The parasite density was done in respondents who had blood slide positive and the majority 169 (65.8%) had parasite density ranging from 40 to 200/µl. All the samples revealed *plasmodium falciparum*. The HRP II antigen detection Paracheck *Pf* for the rapid diagnosis of *falciparum* malaria was used in this study, and out of the total respondents 389, 308 (79.2%) were positive and 81 (20.8%) were RDTs negative, giving a higher percentage of positive results than the microscopy results (table 11), but closer to respondents who presented with signs and symptoms of malaria 342 (88 %). Out of these, the majority 316 (79 %) had fever, and out of those who were RDT positive 308 (79.2%), 265 (86 %) had fever (Table 3 & 7), making fever the core pre-determining sign of malaria after ruling out other illnesses that may cause fever {(MoH, (1999) and Smith T., (1994)}

The sensitivity of RDTs in this study was 96.1% with 95% CI of 93.7 – 98.5, and the specificity was 53.8% with 95% CI of 45.3 – 62.3, Positive Predictive Value was 80.2% and Negative Predictive Value was 87.6 % (Table 11). These results show that the sensitivity is comparable to other study' findings that range between 73.7% and 100% {

100% by Swarthout et al (2007); 98% by Singh Neeru and Saxena Ajay (2005); 91.2% by Guthmann JP (2002); 80.25% by Kaushik Anil, at el (2001); and 73.7% by Tjitra N (2000)}. Results from this study show that the use of Paracheck-*Pf* is almost as sensitive and good as microscopy in detecting true falciparum malaria cases though 3 (0.7%) were found to be false negative. This is paramount, as true malaria cases would be captured and treated on time in this vulnerable population of children 0-15 years of age, and few that are not positive would be treated based on signs and symptoms and microscopy where such a service is available.

While some studies have shown relatively high RDT specificity, this study presents a relatively low specificity of 53.8% which is a bit less compared to other studies that were population based with findings ranging from about 70.75% to 100% {100% by Shiff at el (1993); 91% by Moody, A. (2002); and 70.75% by Kaushik A. at el (2001)} This is mainly because of many cases that came up as false RDT positives {61 (46.2%) of the total RDT positive 308 (79.2) (Table 10)}. However, this study shares same findings with other studies that were clinical based such as Swarthout et al (2007) with 52 %. The study reports the majority of respondents 342 (88 %) (table 3) to have clinical presentations of malaria and this is likely to have increased the chances of capturing HRP II plasmodial antigen with RDT which later increase the RDT false positive. The RDTs have so many advantages in the diagnosis of Malaria for they are able to detect the malaria antigen as long as someone is infected, this study reveals a number of children {61 (46.2%)} who are positive with RDTs and yet negative by microscopy, and a good number of respondents {100 (22.9%)} had parasite density of 40/µl. This is evidence that most of the respondents who are false positive may have had parasite density less that the

threshold level of 50/µl. the Microscopy can miss the parasites due to human errors, inexperience, work overload associated with tiredness, quality of stain and that of microscope, shortage of equipments and takes a long time to come with results, but on the other hand the RDTs requires less expertise, few if no human errors are expected and it only takes 10-15 minutes to come up with results reducing on the work overload and increasing the diagnostic coverage. These findings give reasons to why RDTs should be scaled up so that medications are given only to those that are truly infected and it will also help reduce on the unnecessary misuse of drugs, cuts on expenses and reduce resistance to newly introduced malarial drugs.

In addition, false-positive results of RDTs can occur if patients have undertaken self-medication prior to presentation (Moody, A. 2002), in this case the clinicians are supposed to ask tactically about the clients past medical history and be backed by Microscopy if they are to come up with accurate diagnosis. Apart from the competency of microscopists, it is likely that few of the respondents with false-positive results may have performed self-medication with antimalarial drugs during an attack of fever. However, it is unlikely that these factors account for the entire set of false-positive cases mainly because the researcher excluded all those that were likely to have taken any self medication by asking about their past sickness history and medication taken, and besides about 42 (49.4%) had a history of fever for three to four days (table 13), also only 25(6.4%) (table 3) had a history of a previous illness, and 7 (28 %) of them had false positive results , for all these results related to previous state of health, it gives clients to have had less time to self medicate themselves. It is more probable that most of the false-positive cases were true positives which were not detected by microscopy, due to

sequestration limiting the number of circulating parasites at the time of blood collection or due to the parasitemia being below the detection limit of approximately 50/µl by microscopy as stated by Russell E. at el (2000), and also Bell *et al.* (2005) using PCR, showed that false positive results can be explained by the presence of blood samples with parasite density levels below the detection threshold for microscopy. All these could explain the fact that studies conducted in clinical set up are likely to have a low specificity.

Looking at the study parasite density, it starts from 40µl to 112680µl, the majority of the blood slide positive respondents 169 (65.8%) had parasite density ranging from 40-200/µl (table 10) and those who had parasite density of 40 parasites/µl were 100 (22.9%). This could explain that a good number of false positives may have had less than 50/µl parasite density. An independent expert microscopist was sought and requested to increase the reading fields to more than 500, 10 negative blood slides that were matching with the RDTs positive were randomly selected and re-examined, only 1 (10%) came up positive at a parasite density of 16/µl and this could also explain that more blood slides could have had parasite density below the parasite threshold level. Moreover, in *P. falciparum*, the parasite density may also be difficult to find since they disappear from the peripheral blood after 24-26 hours of asexual development as a result of adherence to infected erythrocytes to the endothelium of venule and capillaries in the vital organs {Schallig, H.D.F.H. & Schoone, G.J. (2003)} . Thus, if peripheral smear is examined after this stage, which could have occurred in this study's samples, it may not detect parasites but the HRP-II test is not likely to miss the diagnosis. This could be another reason for the discrepancy in this study.

The performance of RDTs could have been influenced by the level of parasitemia in peripheral blood, The sensitivity of the Paracheck p.f HRP-II RDTs was 100% at parasitemias above 440/µl; however, the sensitivity dropped from 98 to 50% at parasitemias less than 440 to 40/µl. These study findings are consistent with earlier findings describing that the sensitivity decreases in correlation with the decrease in parasitaemia {Kaushik Anil at el (2001), Guthmann JP at el (2002), Tjitra N at el (2000)}. This can potentially be dangerous, as to miss the diagnosis of malaria in an ambulant patient may mean that complications develop because appropriate treatment was not instituted in time.

The assessment of a negative result in this situation will be clearly influenced by the clinical features, though, study reports 3 (0.7%) of clients who presented with false negative and had no fever, making it more complex and lead to further delay in treatment, increased transmission and malaria complications, therefore, Microscopy will still be needed to confirm the diagnosis.

10. CONCLUSION

The Rapid Diagnostic Tests (RDTs) are rapid and simpler to perform and to interpret by any literate person including Community Health Workers. This shows how desirable these public health tools are to alleviate the diagnostic challenges faced by many people who can not access microscopy services for malaria diagnosis in Zambia. This study results add to the evidence that these non-microscopical rapid tests for the detection of plasmodial antigens may develop into important diagnostic tools and can prove to be a valuable adjunct to clinical assessment of the patient and blood film microscopy under certain circumstances.

The study findings demonstrated that the sensitivity of the RDT was high (96.1% with 95% CI 93.7 – 98.5) and it is comparable to other studies that were done previously. These findings confirm that the RDTs are very effective as diagnostic tools and can be a substitute where laboratory facilities are unavailable.

This study's specificity results (53.8% with 95% CI 45.3 – 62.3) is relatively less compared to other studies that were population based, however, it raises particular evidence that study done in clinical settings are likely to have less specificity of the RDTs HRP-II for Malaria infection and this increases the chances of spending more on unnecessary medication.

The study shows Positive and Negative predictive values of 80.2% and 87.6 % respectively and these findings correlate with other reviewed study findings.

The parasite density shows that the majority of respondents 169 (65.8%) had parasite density ranging from 40-200/µl and the parasitaemia influenced the sensitivity as the higher the parasite density the higher the chances of RDT becoming positive.

The study findings also revealed that there were more false positive {61 (46.2%) of the total RDT positive 308 (79.2%)} which explains the outcome of a relatively low specificity. This was attributed to low parasite density that might have resulted from limitations of microscopy, especially the parasite sequestration limiting the number of circulating parasites in peripheral blood but on the other hand the HRP-II are less likely to be missed by the RDTs. This study clearly demonstrates that the RDTs have the ability (96.1%) to identify the *Pf* antigen in the blood film much faster (15 minutes time) than the microscopic investigation (45 minutes). The use of RDT both for screening and survey purposes where the test can be carried out in 15 minutes time (according to manufacturer instructions) and all positive cases could be identified and treated with an appropriate drug regimen would be very useful in reducing the threat of malaria which is a major public health challenge.

11. **RECOMENDATIONS**

This study revealed a number of issues that need to be worked on and the following recommendations were made:

- Clients are likely to self-medicate themselves and may fear to tell the truth to the health providers and due to such ignorance their RDTs will produce more false positive. Therefore, the need to intensify health education about the effects of previous treatment of malaria on RDTs.
- The Zambia Ministry of Health plans to reduce the malaria burden by 70 to 80 percent through efforts to scale up malaria control interventions. This public health task can not be realised without malaria diagnostic tools. Therefore, the need to scale up the provision of RDTs in all public health facilities.
- More health care providers need to be reoriented and trained on the use of RDTs, for acceptability and compliance in the implementation of these important public health tools. The Paracheck RDT has demonstrated a high sensitivity (96.1%) and so may improve areas that go with use of this tool.

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ANNEX I

INFORMATION SHEET

Project title: Effectiveness of Rapid Diagnostic Test (RDT) for Malaria Diagnosis in Children under 15 years of Age of Nchelenge District.

Name of the Principal Investigator: Ndayambaje Israel

Institution of the principal Investigator: University of Zambia, School of Medicine, Community Medicine.

Affiliation/Status of the Principal Investigator: Student of Masters in Public Health

Purpose of the study

We invite you to participate in a study of comparing the use of microscopy and a newly developed diagnostic tool named Rapid Diagnostic Tests (RDT) for Malaria detections. The objective of this study is to come up with strengths and weakness of the RDT test (an alternative to microscopic examination) in patients having malaria. This tool is very simple to use and once it is found to be efficient it will contribute to gaps experiencing by health facilities in terms of malaria diagnosis. Recommendations will also be given to policy makers.

Procedures

Specifically we are going to ask you few questions about you becoming sick of malaria and if you have had any intervention of malaria before. The interview will take less than 15 minutes and we will take some blood of your finger prick to test for malaria.
The information that you will provide during the study will be kept confidential. Only the interviewer and researcher will have access to the questionnaires and malaria results. The information will be destroyed after the study.

Benefits

By participating to this study and answering to our questions you will help to increase our understanding of the usefulness and efficiency of RDT tests to the community. And if you are found malaria positive you will access appropriate malaria drugs.

Your participation to this **study is voluntary** and you have the **right to refuse to participate or to answer** to any question that you feel comfortable with. If you change your mind, you have the **right to withdraw at any time** and whether you participate in the study or not you still have the **right to access malaria medication** if you have malaria.The procedure of taking blood on your finger prick is slightly painful and if you do not feel comfortable to give blood for test you are free to say no. If anything is not clear or if you need further information, we shall provide it to you.

Confidentialities

All information collected throughout this study will be kept confidential. However, the details resulting from this study will be used to complete this report and be disseminated to NMCC and MoH.

CONTACT PERSON:

Please fell free to contact the persons below at anytime if you have any questions about participating in this study:

The Chairperson
UNZA Research Ethics Committee,
PO BOX 50110,
Lusaka,
Zambia.
Telephone: 260-021-1-256067
E-mail: unzarec@zamnet.zm

The Principal investigator,
Israel Ndayambaje
Master's Student in Public Health
C/O UNZA, School of medicine,
Community Medicine Department,
PO Box 50110
Lusaka,
Zambia.
Mobile No: 097-7-530706
E-mail: israelnam@yahoo.co.uk

CONSENT FORM

Declaration of the Respondent (A Parent or guardian).

I have understood that the aim of the study is to examine the effectiveness of RDT compared to microscopic examination. I realise that I will have to answer few questions and give some finger prick blood for malaria testing.
I consent voluntarily to participate in this study.

Signature of Client: ..... Signature of Interviewer.....

Date: ---------- Date:----------

Annex II

STRUCTURED INTERVIEW SCHEDULE FOR RESPONDENTS

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

DEPARTMENT OF COMMUNITY MEDICINE

DATE OF INTERVIEW: .....

RESPONDENT'S NUMBER: .....

NAME OF THE CLINIC: .....

INSTRUCTIONS TO INTERVIEWER

1. Introduce yourself to the respondent.
2. Explain the purpose of interview.
3. Reassure the respondent that all responses will be held in strict confidence.
4. Read information sheet to the Respondent, followed by the consent/assent form.
5. Individual names and address should not appear on the interview schedule.
6. Ensure that all questions are answered and indicate response by ticking in the appropriate box or filling in appropriate spaces provided.
7. Thank the respondent at the end of interview.

Cover all questions and tick or write in the corresponding Box and fill in the blanks

1. Name of a Clinic/Hospital: ..... Respondent number:.....

2. How old is your child? ..... (write years or Months if less than one year)
3. Sex: Boy ..... Girl ..... (tick the appropriate response)

4. In which village are you staying.....
5. Any signs and symptoms of Malaria:
    a. Yes.....
    b. No.....

6. State signs and Symptoms your child is presenting with:
.....
.....
7. Is he/she clinically diagnosed with malaria? (Interviewer to notify from the card)
    a. Yes.....
    b. No.....

(If No to Q5 & 7, Go to Q 16)

8. Has he/she been ill with fever at any time in the last 2 weeks?
    a. Yes.....
    b. No.....

9. How many days ago did the fever start?
    a. Days.....
    b. Don't know.....

10. Was your child diagnosed malaria parasite positive.....
11. How many days ago did other signs and symptoms (if any) of malaria start?
    a. Days.....
    b. Don't know.....
12. At any time during the illness, did the child take any treatment?
    a. Yes .....
    b. No .....

13. If Yes, where ?: Public sector (Government hospital, clinic or field worker) .....
Private medical sector (Pharmacy, Surgery) .....
Traditional practitioner .....
Other sources (Shops, Friends) .....

14. What drugs were you given.....
    a. (If he/she does not know, write Don't Know)

15. Does your child still have fever or other signs and symptoms?
    a. Yes .....
    b. No .....

16. Any history of fever/other signs and symptoms of malaria before the onset of the current disease?
    a. Yes .....
    b. No .....

17. If yes, how many days have passed? .....

Annex III.

EFFECTIVENESS OF RDT FOR MALARIA DIAGNOSIS

LABORATORY RESULTS FORM

Respondent Number .....

Laboratory Tests:

RDT		**MICROSCOPY (Indicate positive or Negative)**		
		1st Lab Technician	2nd Lab Technician	3rd Lab Technician
Positive				
Negative				
Invalid				

Other Microscopic Results:

MICROSCOPY	Type of Plasmodium	
	Parasite density - No. of Parasites per Microlitre present	

Annex IV

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@zamtel.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

27 February, 2008
Ref.: 011-10-07

Mr Israel Ndayambaje, BSc Nursg
Department of Co1 .munity Medicine
School of Medicine
University of Zambia
LUSAKA

Dear Mr Ndaya..baje,

RE: RESEARCH PROPOSAL: **"EFFECTIVENESS OF RAPID DIAGNOSTIC TEST (RDT) FOR MALARIA DIAGNOSIS IN CHILDREN UNDER 15 YEARS OF AGE OF NCHELENGE DISTRICT"**

The above research proposal was presented to the Research Ethics Committee Secretariat meeting held on 19 October, 2007, where changes were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal has now been approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.

Yours sincerely,

Dr E. Munalula Nkandu, BSc (Hons), MSc, PgD R/Ethics, PhD
CHAIRPERSON

Date of approval: **22 February, 2008** Date of expiry: **21 February, 2009**

Annex V

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF COMMUNITY MEDICINE

Telephone: 252641,
Fax: + 260-1-250753,

P.O. BOX 50110,
Lusaka, Zambia.

==========

15th August 2007.

The Director,
St. Pual's Hospital,
NCHELENGE.

Dear Sir/Madam,

RE: **REQUEST FOR PERMISSION FOR MPH STUDENT TO COLLECT INFORMATION FOR RESEARCH.**

We are writing to kindly request for permission for Mr. Israel Ndayambanje who is currently studying for his Masters in Public Health (MPH) to collect information at your office. The collected information would serve the purpose of writing his Research on:

"Effectiveness of Rapid Diagnostic Tests for Malaria Diagnosis in Children Under 15 year of Age of Nchelenge District".

We appreciate your support to our MPH programme and the student.

Yours Faithfully,

Mr. T Glover-Akpey
MPH COORDINATOR.

DEPT. OF COMMUNITY MEDICINE
SCHOOL OF MED
UNIVERSITY OF
P.O. BOX 50110, LUSAKA.

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF COMMUNITY MEDICINE

Telephone: 253641,
Fax: + 260-1-250753,

P.O. BOX 50110,
Lusaka, Zambia

15th August 2007.

The District Director,
Nchelenje DHMT,
NCHELENGE.

Dear Sir/Madam,

RE: REQUEST FOR PERMISSION FOR MPH STUDENT TO COLLECT INFORMATION FOR RESEARCH.

We are writing to kindly request for permission for Mr. Israel Ndayambanje who is currently studying for his Masters in Public Health (MPH) to collect information at your office. The collected information would serve the purpose of writing his Research on:

"Effectiveness of Rapid Diagnostic Tests for Malaria Diagnosis in Children under 15 year of Age of Nchelenge District ".

We appreciate your support to our MPH programme and the student.

Yours Faithfully,

Mr. F. Glover-Akpey,
MPH COORDINATOR.

DEPARTMENT OF COMMUNITY MEDICINE
SCHOOL OF MEDICINE
UNIVERSITY OF ZAMBIA
P. O. BOX 50110, LUSAKA

Annex VI

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH

Nchelenge District Health Office, P.O. Box 540017 Nchelenge, Tel/fax 972077, Tel 972072

22nd August 2007

The M.P.H Coordinator (Mr. I. Glover – Akpey)
Department of Community Medicine
School of Medicine
University of Zambia
P.O. Box 50110
Lusaka

Dear Sir

RE: REQUEST FOR PERMISSION FOR MPH STUDENT TO COLLECT INFORMATION FOR RESEARCH

I refer to your letter dated 15th August 2007 in which you requested for permission for M.P.H student to collect information for research.

The District has no objection for the M.P.H. student to collect information for research,Mr. Israel Ndayambase has been granted permission to conduct research on effectiveness of R.D Tests for Malaria Diagnosis in Children under 15 years of age of Nchelenge District.

The District would be very happy to get a copy of the results, after the exercise.

Yours faithfully

O.S. Kalembwe
MANAGER ADMINISTRATION

FOR DISTRICT DIRECTOR OF HEALTH

ST. PAUL'S MISSION HOSPITAL

P.O. BOX 740106 NCHELENGE – ZAMBIA

TEL: 02 972078 FAX: 02 972108

Email: stpaulshosp@zamnet.zm

27 August 2007

The MPH Co-ordinator
University of Zambia
Department of Community Medicine
P O Box 50110
LUSAKA.

Dear Sir,

RE: COLLECTION OF INFORMATION FOR RESEARCH MPH STUDENT – ISREAL NDAYAMBAJE

Thankyou for the request made to our Institution to allow Mr. Isreal Ndayambanje carryout his research project using our facilities.

We have no objections and we stand ready to offer him any assistance needed, in facilitating his project.

Yours faithfully,

Sr. Mwale...

Sr. Honesta Tembalamba
HOSPITAL ADMINISTRATOR
*For*HOSPITAL MANAGEMENT TEAM.