EVALUATION OF DIAGNOSTIC PERFORMANCE AND FACTORS AFFECTING MALARIA RAPID TEST KITS IN SEME, KISUMU COUNTY

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DECLARATION

This thesis is my original work prepared with no other than indicated sources and support has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled "**Evaluation of Diagnostic Performance and factors affecting Malaria Rapid Test Kits in Seme, Kisumu County".**

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COPYRIGHT STATEMENT

DEDICATION

This work is dedicated to my parents, Mr. and Mrs. Okuta and my husband Daniel Ochola for their continued support throughout my study.

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ABSTRACT

Malaria disease is a global challenge and a major public health threat. Quality diagnosis of malaria is a key aspect of health care, as it provides an explanation of patient's health problem and informs subsequent health care decisions. Microscopy is the reference test method for malaria diagnosis; however, the availability and utilization rate of malaria rapid diagnostic tests (mRDTs) has increased with time. World malaria report documents the evidence of gene deletion of plasmodium falciparum histidine rich protein 2 and 3 genes (*pfhrp2/3*), rendering malaria parasite undetectable with HRP2 based mRDTs that are commonly available. This may cause delay in treatment, misdiagnosis and in some cases death, hence the need to evaluate the diagnostic performance of mRDTs and the factors affecting them. The aim of the study was to evaluate the diagnostic performance and determine factors affecting mRDTs (histidine-rich protein2 (HRP2) and HRP2 combine with parasite lactate dehydrogenase (HRP2/pLDH) RDTs. Cross-sectional analytical study design was conducted in three purposively sampled health facilities (Ratta, Miranga and Manyuada) in Seme Sub County. The facility selection was based on workload, prevalence and the diagnostic methods used. Cochran formula was used to calculate the sample size. Convenience sampling was used to select the health care workers interviewed and a total of 223mRDTs each were randomly selected. Microscopy was used as a reference method to demonstrate the diagnostic performance of the different mRDTs using 223 whole blood sample panels collected from the patients suspected to have the disease. Structured questionnaire was administered to health care workers to assess the factors affecting mRDTs. Blood collections from the participants were done by a trained phlebotomist to minimize harm. Data analysis was done using Cohen Kappa Coefficient, MCNemar's test, likelihood ratio, receiver operating characteristic (ROC) and descriptive statistics used for objective three. Presentation was done in charts, figures and tables. The results were as follows; for HRP2 RDTs, sensitivity (99.4%), specificity (85.7%), positive predictive value (81.0%) and negative predictive value (96.0%) and for pLDH/HRP2RDT,sensitivity (94.4%), specificity (85.0%), positive predictive value (80.2%), and negative predictive value (96.0%).HRP2 RDTs and microscopy gave a significant perfect agreement of (*P*-value<.001) and between microscopy and pLDH RDT of (*P*-value<.001).However, there was a significant disagreement between HRP2 and microscopy of *P*=.004 and HRP2/pLDH of P=.002. Further results revealed that both the mRDTs had minimum parasite density detection limit of 80 parasite/µl of blood and the Factors affecting the performance of mRDTs were as follows, 47% of health facilities had appropriate storage area, 18% of the health providers were able to checked and monitor the expiry dates, 39% reported availability of malaria commodities. Training for the health care workers in this study were below 60%. There was no evidence of internal and external quality control in place. In conclusion, the agreement between malaria positivity using microscopy and mRDTs positivity increased with increase in parasite density, however the mRDTs performance was below the expected world Health Organization expectation of ≥ 95%. Storage, quality assurance, end user training and commodity stock level monitoring in health facilities in Seme sub county were the factors affecting the performance of mRDTs. The study therefore advocates for more sensitive kit that can detect parasite at low density level for future use, especially to improve the sensitivity of mRDTs kits in malaria management where trained microscopist for malaria diagnosis are not available. Temperature monitoring of the storage areas for MRDTs, training of staff, quality assurance measures and proficiency testing is recommended for the effective performance of mRDTs.

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LIST OF ABBREVIATION AND ACRONOMY

EBLM	Evidence Based Laboratory Medicine
EBM	Evidence –Based Medicine
EDTA	Ethylene diamine tetra acetic Acid
IERC	Institutional Ethical Research Committee
LDH	Lactate Dehydrogenase
MOH	Ministry of Health
MRDTs	Malaria Rapid Diagnostic Tests
NACOSTI	National Commission for Science, Technology & Innovation
pLDH	Parasite Lactate Dehydrogenase
RBCs	Red blood cells
RDTs	Rapid diagnostic tests
SOPS	Standard operative procedures
W.H.O	World Health Organization

OPERATIONALIZATION OF TERMS

Diagnostic performance is the ability of a qualitative test to detect a condition when it is present and detect the absence of a condition when it is absent. It measured by the sensitivity, specificity and predictive values of a diagnostic test kit.

Evaluation is the process by which a test kit is compared against reference test method and specifications through testing. The results are evaluated to assess performance.

Immune chromatography assay is the immunological binding assay, employs paper chromatography to separate immune complexes from free antigen and antibodies.

Monoclonal antibodies this is an antibody produced by a single clone of cells or cell line and consisting of identical antibody molecules.

Test validity can be described as the extent to which a test accurately measures what it is supposed to measure.

Specificity (the true negative) rate measure the proportion of actual negatives that are correctly identified as such.

Sensitivity (the exact positive rate), this is the recall or probability of detection measures that proportion of exact positives that are correctly identified as such.

Predictive value the positive and negative predictive values (PPV and NPV) are-the proportion of positive and negative results in diagnostic tests that are true positive and negative results, respectively. PPV and NPV describe the performance of diagnostic tests or other statistical measures.

Gold standard is the diagnostic test or benchmark that is the best available under reasonable condition

Parasite density is the quantity of asexual stage of malaria parasites counted per microliter of blood (Parasite/µl of blood).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information of the study

Malaria is a parasitic disease caused by a protozoan of the genus *Plasmodium* and is spread by female *Anopheles* mosquitoes (Amoah *et al.,* 2019). It is the highest contributor to morbidity and mortality in the world's developing countries (Kapesa *et al.,* 2018).*Plasmodium falciparum* causes a more significant proportion of malaria disease in Africa (Dalrymple *et al.,* 2017), which also contributes to the highest death rates in children under five years of age (Weiss *et al.,* 2019).

The recent world malaria report released in December 2019showedthat, about 200 million malaria cases in 2018 below against to 2.3 million patients in 2018. The approximated malaria deaths was estimated at about 400,000 in 2018, compared with 416 000 deaths in 2017 (Lee *et al.,* 2018).There is a high share of the global malaria burden in the African region and it accounted for 93% and 94% of the worldwide malaria morbidity and mortality, respectively in 2018. Kenya reports four million malaria cases annually. 80-90% of malaria cases in Kenya are attributed to *P.falciparum*, even though *P.malariae*, *P.ovale* also exist in the county (Kane, 2019). In Kenya malaria causes a significant public health risk and it remains the leading cause of morbidity and mortality in the county (Githinji *et al.,* 2017). Approximately 6.7 million new clinical infections are recorded yearly, with about 4000 deaths occurring, majorly, among children hence making malaria a significant health burden for Kenya (Kirinyet, 2019).

Microscopy is the ‘gold standard’ accepted for malaria diagnosis and is regarded as the reference test method (Berzosa *et al.,* 2018). However, to minimize challenges associated with microscopy, various types of RDTs kits have been manufactured to diagnose malaria in non-endemic and endemic zones as part of malaria diagnosis (Mukkala *et al.,* 2018). The quality of these malaria rapid diagnostic kits has improved with time(Adu-Gyasi *et al.,* 2018), however, there is still need to evaluate the diagnostic performance and determine the factors affecting these test kits in comparison to the existing reference test method.

In Sub Saharan Africa, the mRDTs have the highest distribution rate of 66% and accounted for 76 % of all the diagnostic test done in 2017 (Briand *et al.,* 2020; Echodu *et al.,* 2020). This indicates that they have displaced conventional microscopy in many endemic areas as a standard practice. The mRDTs commonly available and used worldwide are based on the detection of parasite Histidine-rich protein 2 (PfHRP2) gene (Krause *et al.,* 2018). Histidine-rich protein 2 (HRP2) antigen incorporated in most mRDTs are encoded by *hrp2*gene. Spontaneous *hrp2*deletions are documented to happen, as was seen by (Berhane *et al.,* 2018) and also reported by World malaria report 2020. *P.falciparum* parasites demonstrating gene deletions cannot be detected using mRDTs targeting HRP2 proteins (Beshir *et al.,* 2017). Therefore, mRDTs based solely on HRP2 would give false-negative results when applied to diagnose patients infected with malaria parasite strains with *hrp2* gene deletions. To improve malaria diagnosis and overcome the false negative in patients infected with parasites harbouring *hrp2*-deleted gene, mRDTs that combine multiple parasite protein targets have been developed. Parasite lactate dehydrogenase (pLDH) is one of these target proteins that can be used to detect plasmodium parasite with *hrp2* gene deletion. Studies indicate that adding pLDH to HRP2 in mRDTs can enhance their ability in detecting malaria parasites,

including those with *hrp2-gene* deletion (Adu-Gyasi *et al.,* 2018; Kozycki *et al.,* 2017a). There are several causes of false-negative (FN-RDT) results (Berhane *et al.,* 2017). First, the parasiteamia level may be below the RDTs quantitative limit of detection (LOD), typically in the range of 200 parasites/µl of blood (De Koninck *et al.,* 2017),Which may differs, sometimes even within the same geographical region, as seen in Angola (Plucinski *et al.,* 2019). Second, other *Plasmodium* species cannot be demonstrated by the commonly used PfHRP2-based RDTs (Berhane *et al.,* 2018). Third, poor storage of mRDTs, including prolonged exposure to extreme temperature conditions, can impair mRDT performance (Watson *et al.,* 2019). Fourth, a technical inaccuracy can result if the mRDT test lines are misread or if the result is interred before or after the recommended testing period. Finally, lot-to-lot variation can influence mRDTs diagnostic accuracy (Organization, 2016). More studies on factors influencing the performance of mRDTs are important has recommended in an exploratory study done in Ghana (Opoku, 2018) and Moonasar. This study is to follow up on these recommendation.

In Kenya, HRP2 mRDTs are the commonly available mRDTs (Nderu *et al.,* 2018) and the brands available in Seme Sub County that were used in this study are SD BIOLINE malaria Ag P.f (lot 05FK50) manufactured by Abbot diagnostic in Korea and ParaScreen ® rapid test for malaria pan/pf (lot 101343) manufactured by Zephyr Bio- medicals in India. Several factors affects the accuracy of mRDTs and these includes; manufacturing defects, storage, transport and end-user technical skills (Usman *et al.,* 2018). Operational issues relating to performance and factors affecting mRDTs and their use have not been carefully investigated. This study was therefore done to evaluate the diagnostic performance and determine factors affecting malaria rapid diagnostic testing in Seme Sub County, Kisumu County.

1.2 Problem Statement of the study

Malaria disease is one of the global life-threatening diseases and Kisumu county prevalence rate was at 19% in 2021. Among the available diagnostic methods, rapid testing is inexpensive and can be done in remote. Kisumu County has adopted the strategy of mRDTs in all the seven sub-counties with Seme recording the highest utilization rate of 76%. The types used are HRP2 and HRP2/pLDH antigen based mRDTs. The manufactures of these mRDTs documents that the sensitivity to be is 100% with >50 parasite/µl of blood. This will lead to missed opportunity which may impair treatment and prevention strategies. Hence the need to determine the sensitivity, specificity and predictive values and the exact quantitative limit of parasite density cut off point of these mRDTs? The mRDTs can be affected by prozone effect and may cause false negative result and also produce a false positive result for patients with acute schistomiasis. And being a biological agent, they can also be affected with other factors not clearly known. The WHO malaria report of 2020 documents evidence of deletion in the *pfhrp2* and *pfhrp3* (*pfhrp2/3*) genes also hindering parasites detection by mRDTs HRP2, making these mRDTs unreliable (Grignard *et al.*, 2020).This might lead to misdiagnosis of malaria through generating false-negative results which might end up delaying malaria treatment and in worst cases cause mortalities. This therefore calls for the need to evaluate the diagnostic performance and factors affecting the malaria rapid diagnostic test kits.

1.3 Objectives of the study

1.3.1 Broad objective

To determine the diagnostic performance and the factors affecting malaria rapid diagnostic tests.

1.3.2 Specific objectives

i. To determine sensitivity, specificity, and predictive values of HRP2 and HRP2/pLDH based mRDTs in Seme, Kisumu county

ii. To estimate the quantitative limit of parasite density cut off points detected by the different mRDTs

iii. To assess factors affecting the performance of malaria RDTs in Seme Sub County

1.4 Research Questions

i. What is sensitivity, specificity, predictive values of HRP2 and HRP2 / pLDH based RDTs in Seme, Kisumu County?

ii. What is the quantitative limit of parasite density cut off points detected by the different RDTs in Seme, Kisumu County?

iii. What are the factors affecting the performance of malaria RDTs in Seme, Kisumu County?

1.5 Justification of the study

Malaria remains to be a major burden and the number one out of the ten diseases given high priority in Seme Sub County. Quality malaria diagnosis, treatment and prevention are dependent on the application of the right diagnostic technique. World health organization recommendation on parasite-based diagnosis in regions where expert microscopy is not available can easy be achieved by use of rapid diagnostic test. It is important to determine whether these tests are useful in a specific endemic region, as well as the most appropriate mRDTs to use and the factors affecting their performance.

Microscopy is an established, relatively simple technique that is familiar to most laboratory officers. It can determine that malaria parasites are present in the patient's blood, determine the malaria species and the parasitemia level. High-quality malaria microscopy is not always immediately available in every clinical setting where patients might seek medical attention. These practices have resulted in long delays in diagnosis. The facilities not able to use microscopy may now use mRDTs to more rapidly determine if their patients are infected with malaria. These mRDTs like other biological test are more prone to deterioration through exposure to unfavorable conditions. Data on the diagnostic performance and factors affecting the performance of rapid diagnostic test device is important information to lead in diagnostic test selection by the relevant authorities.

1.6 Significance of the study

World health organization indicates that the role of laboratory diagnosis is to assist malaria case management focusing on the use of rapid diagnostic tests in areas endemic for malaria disease. For this recommendation to be adhered to, the malaria control unit should establish fast and accurate laboratory findings or malaria parasite demonstrations. The findings from the study will help the Ministry of Health and the Malaria control unit to ensure accurate, reliable and timely diagnostic method for malaria is used. Promote the availability of adequate competent medical laboratory officers in all health facilities and refresher training on malaria case management done to them. The findings will facilitate effective monitoring of mRDTs during transportation, storage and test performance and finally to enhance the availability and utilization rate of standard operating procedures and WHO guidelines on malaria diagnosis. Accurate, reliable and timely diagnosis will be cost-effective to the patients

by reducing their frequent visits to the hospitals and finally reduce morbidity and mortality resulting from malaria disease.

1.7 Scope of the study

The purpose of the study was to evaluate the performance of rapid diagnostic tests using the reference test method. The study also determined the factors affecting the diagnostic accuracy of these rapid diagnostic tests. The suspected patients referred to the laboratory for malaria test were bleed and there blood samples formed the sample panels for mRDT kits evaluation. A total of 223 sample panels each were subjected to the three tests, microscopy, HRP2 mRDTs and HRP2/pLDH mRDTs. A category of health care workers were interviewed for factors affecting the diagnostic performance of mRDTs. The study was carried out for three months where diagnostic performances of the kits were assessed. The study area was Seme Sub County and three facilities (Ratta, Miranga and Manyuada) were targeted.

1.7.1 Limitations

The limitation of this study was that the known blood bank samples could not be obtained to be used to evaluate the kits. We recommend future studies be done on how to obtain blood bank samples that can be archived to support such studies. However, this is still challenging because whole blood samples recommended for malaria diagnosis have a short shelf life. Another limitation was that the malaria diagnostic test kits evaluated were from the same manufactures and similar lot (batch) number and expiry date. In future, there is a need for manufactures to think of producing different batches to allow the batch to batch evaluation to be possible.

1.7.2 Delimitations

This study does not cover the entire Seme Sub County health facilities; it covers only three high volume sites. Data collection took three months to enable us to achieve the study objectives. The population size which forms the source of the blood samples used to evaluate the diagnostic test was obtained from the patients suspected to have malaria and are sent to the laboratory for a malaria diagnostic test.

1.8 Theoretical model

The study adopted Evidence-Based Medicine (EBM) which is the idea that occupational practice ought to be based on scientific evidence (Thomas *et al.,* 2011).It aims to provide the most effective care that is available, with the aim of improving patients' outcomes. Patients expect to receive the most effective care based on the best available evidence. The Evidence-based laboratory medicine(EBLM) is a separate branch of EBM which focuses on the evaluation and use of laboratory tests with an overall aim of improving patient's outcomes (Chin *et al.,* 2020). In this theory, a clinician requesting an investigation had a question and needs to make a decision. The clinician hopes that the test results will help to answer the question and assist in making the decision (Diagnostic accuracy).

Price defines EBLM, developed from the definition of EBM (Price, 2012) as 'the conscientious, judicious and explicit use of best evidence in the use of laboratory medicine investigations for assisting in making decisions about the care of individual patients. It might also be expressed more directly in terms of health outcomes as "ensuring that the best evidence on testing is made available, and the clinician is assisted in using the best evidence to ensure that the best decisions are made about the care of the individual patient and that the probability of improved health outcomes increases (Price, 2012).The real effect of diagnostic testing on patients outcomes and

how the effect can be assessed is the concern of EBLM theory (Price, 2012). The practice of evidence-based laboratory medicine seeks to answer three key questions, 1. Is it a quality test? 2. If the test is applied properly will it improve patient outcomes? And 3. Is it worth investigating the test? The theory further explains that a good test is one that is accurate and precise which is generally known as diagnostic accuracy. This concurs with Fryback concept that has been adopted as a conceptual framework in this research. This will enable us to meet the study independent variables. It recommends that decisions are based on the best available, current, valid and relevant evidence from research, informed by clinical expertise and patient values. The components of evidence-based practice includes; Formulating question, Searching for evidence, Appraising the evidence, Applying the evidence and Assessing the experience (Badrick, 2013), all are translated into daily clinical and laboratory practice.

If EBLM principle is combined with routine practice, it will help to resolve some issues such as test cost versus laboratory-generated income and results in an impact on outcome versus test results which is a key indicator to clinical decision making. The issues will be solved by detecting (a) where medical laboratory science fits into the care pathway (b) where diagnostic test is necessary (c) the nature and quality of evidence required to demonstrate the clinical implementation (e) where changes in the treatment flow will occur (f) where benefits of the patients can be achieved.

The practice of EBM can be summarized that "An evidence-based practitioner must understand the patients' circumstances or predicament, identify knowledge gaps and frame questions to fill gaps. conduct an efficient literature search, critically appraise the research evidence and apply that evidence to patient care as explained by Guyatta and colleagues (Law *et al.*, 2008). Hence, the use of this theory in this study.

1.9 Conceptual framework

The research adopted a conceptual framework developed from radiology for evaluating imaging technologies to evaluate laboratory test. The framework addresses diagnostic efficacy, diagnostic effectiveness, therapeutic efficacy and therapeutic effectiveness. These answers the questions being raised by EBLM theory. This conceptual framework was proposed by Dennis Fryback and Jack Thornbury (Silverstein *et al.,* 1994).

The Fryback and Thornbury frame work for diagnostic efficacy addresses six hierarchical levels; Technical efficacy (does the laboratory measure the substance it purports to measure), diagnostic accuracy efficacy (describes the yield- sensitivity, specificity and positive and negative predictive values of a laboratory test in clinical settings), Diagnostic thinking (addresses the issue of whether the diagnostic test changes the thinking of the clinician), therapeutic efficacy (addresses whether a test result changes the clinical management of the patient), patient outcome (describes the outcomes that patients actually experience) and societal efficacy (addresses issues such as the health of the population and the cost of test. Figure 1.1, illustrate the conceptual framework build up from the EBLM theory.

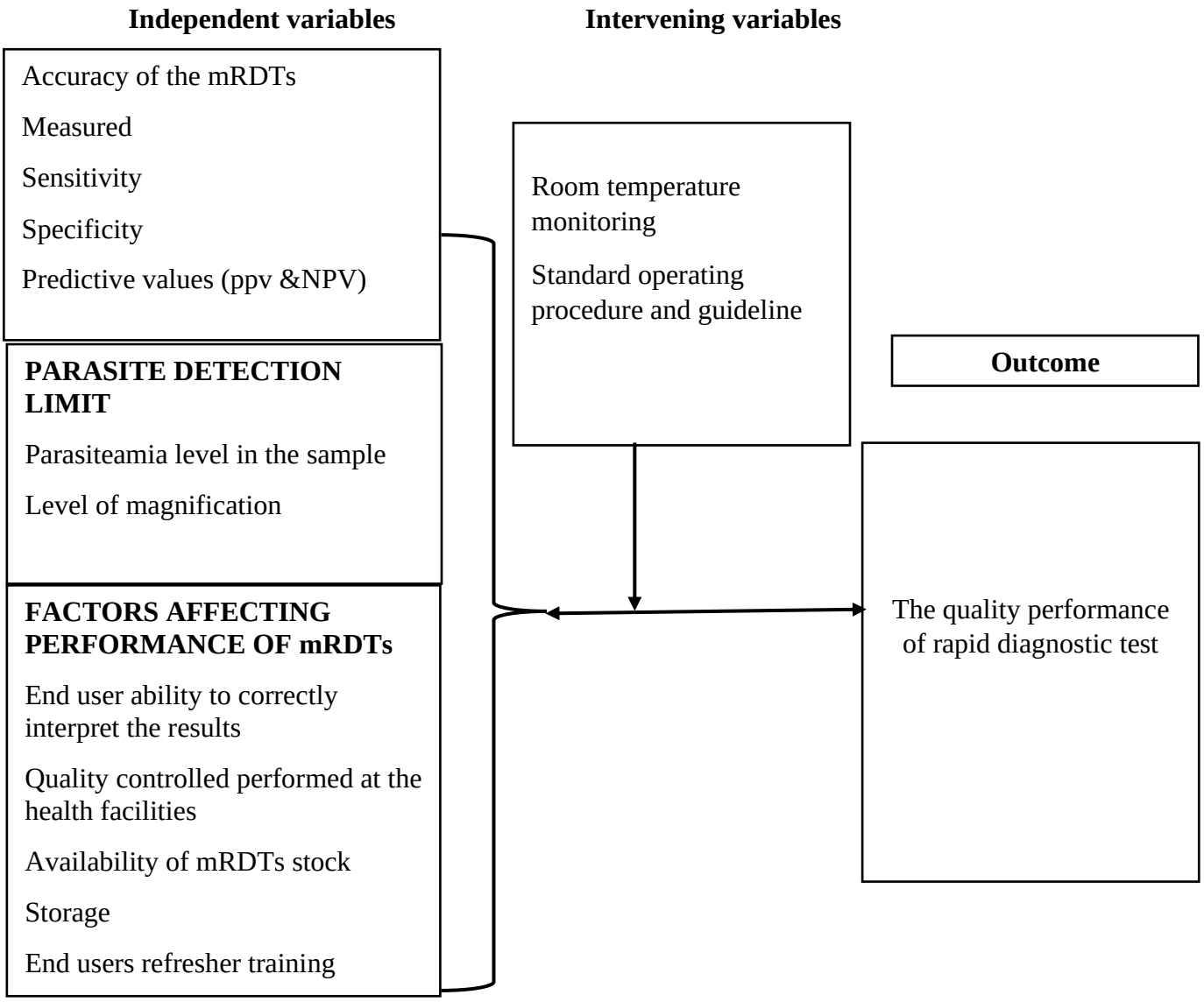


Figure 1.1 Conceptual frame work
Source: Okuta, 2020

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The burden of Malaria Disease

More than three hundred million severe diseases are caused by malaria and it is killing at least one million people yearly (Di Gennaro *et al.*, 2020).There were approximately 2.2 million cases of malaria in 87 countries in 2017. Approximately 435 000 malaria deaths were documented in 2017. Disproportionately high share of the global malaria burden is found in African region (Agbenyega, 2019). In 2017, the African region was home to 92% of all malaria cases and 93% of all malaria deaths. In 2017, the total funding for malaria control and elimination was approximated at US dollar of 3.1 billion. Of this, US dollar 900 million were contributions from governments of endemic regions, accounting for 28% of total funding (Haakenstad *et al.*, 2019). Malaria is the major cause of morbidity and deaths in Kenya, with more than 70 percent of the population at risk (Kapesa *et al.*, 2018). The malaria burden in Kenya depends on the region with the areas around Lake Victoria and the coastal regions having the highest risk, and children under age five and pregnant women are the mostly affected (Okiro *et al.*, 2020).

The clinician's physical examination cannot easily distinguish malaria from other tropical diseases, which are several and can cause potentially harmful effects, but treatable if appropriate management is given early enough. Even in endemic areas with high malaria transmission, other treatable acute infections can cause significant morbidity and mortality (Verjee, 2019). Therefore, it is vital to have an early and accurate diagnosis of malaria to distinguish it from other febrile illnesses early to allow prompt and appropriate treatment of all causes of fever. Proper diagnosis reduces

morbidity and mortality rates resulting from malaria infection and minimizes the wastage of antimalarial drugs. Obtaining an accurate diagnosis gives specific figures on malaria incidence. It enables the following up of malaria disease and targeting antimalarial resources to areas of greatest need and precise evaluation of the impact of interventions. Malaria transmission in endemic zones, is so severe to the extent that a good percentage of the population is infected but do not show clinical symptoms (Jiram *et al.,* 2019). The carriers have developed enough immune response to protect them from malarial illness, but not an infection. In such situations, the presence of malaria parasites in an ill person blood sample does not necessarily mean that it is caused by a recent parasites infection. Lack of resources is a significant barrier to quality diagnosis in many malaria endemic countries. Quality malaria diagnosis if embraced facilitates alleviation of suffering but also minimizes community transmission (Sori *et al.,* 2018).A study done on the prevalence of malaria parasites in adults and its determinants in malaria endemic area of Kisumu County, in Maseno division this was covering Seme Sub County which was the current study side. This study indicated that adult prevalence of malaria parasites in Maseno was 28% (Jenkins *et al.,* 2015) indicating high prevalence of malaria disease in the study area and accurate diagnosis will be very key component of effective treatment and preventive measures.

2.2 Morphology and life cycle of the malaria parasite

Plasmodia has two host life cycle and passes through many stages. The sexual phase occurs in the female anopheles' mosquitoes, whereas the asexual stage occurs in the vertebrate host. The parasite's sporozoites stage is released by sexual reproduction in the midgut of vector female *Anopheles* mosquitoes and migrates to the salivary gland.

Sporozoites are injected into small blood vessels when an infected female *Anopheles* mosquito takes a blood meal from humans (Howick *et al.,* 2019). Sporozoites enter liver parenchymal cells within 30 minutes of injection. In the liver cell, the parasite develops into a spherical, multinucleate liver-stage called schizont, which contains lancet-shaped sporozoite with an approximate size of 1 × 7 μm 2,000 to 40,000 uninucleate merozoites. The process of enormous amplification of the plasmodium parasite is called exoerythrocytic schizogony. This exoerythrocytic or liver phase of the disease usually takes between 5 and 21 days, depending on the *Plasmodium*' species. However, in *P vivax* and *P. ovale* infections, liver-stage schizonts' maturation may be prolonged for 1 to 2 years. These quiescent liver-phase parasites are called hypnozoites. Regardless of the time required for development to occur, the mature schizonts eventually rupture, releasing thousands of uninucleate merozoites into the bloodstream. Each merozoite can attack a red blood cell. The merozoite develops to form either an erythrocytic-stage (blood-stage) schizont (by the process of erythrocytic schizogony) or a spherical or banana-shaped, uninucleate gametocyte within the red cell. The mature erythrocytic-stage schizont contains 8 to 36 merozoites, measuring about 5 to 10 μm long, and are released into the bloodstream when the schizont matures and ruptures. The merozoites released proceeds to infect another generation of erythrocytes in the blood. The time required for erythrocytic-schizogony determines the interval between the releases of successive generations of merozoites-varies with the types of *Plasmodium* and is responsible for the classic periodicity of fever in malaria (Abbas *et al.,* 2019).

Gametocyte, which is the sexual stage, infects mosquitoes that ingest it while feeding on a blood meal. Within the mosquito, gametocytes develop into female and male gametes (macrogametes and microgametes respectively), which undergo fertilization

and then grow over 2 to 3 weeks into sporozoites infect humans. The delay between infection of a mosquito and sporozoites' maturation means that female mosquitoes must live a minimum of 2 to 3 weeks to transmit malaria. This fact is essential in malaria control efforts (Cowman *et al.*, 2016).

2.3 Causative agents, pathogenesis and Clinical Presentation of Malaria

Malaria results from a single-celled protozoan parasite of the genus *Plasmodium.* Four different plasmodium parasite species is capable of causing infection to humans by entering the blood system when a female *Anopheles* mosquito takes a blood meal. The four species includes; *Plasmodium falciparum*, *Plasmodium vivax*; *Plasmodium ovale*; and *Plasmodium malariae* (Zambare *et al.*, 2019).

The development of severe malaria may result from mixed reactions of parasite-specific factors. Such factors include adherence and sequestration in the vasculature and the release of active biomolecules, together with host inflammatory immune reactions. Which include cytokine and chemokine production and cellular infiltrates (Rénia *et al.*, 2016). Cerebral malaria is clinically presented by diffuses symmetrically to encephalopathy with high temperature and absent or few central neurological signs. Fever rapidly can develop within a mean of two days in children (Luzolo *et al.*, 2019). In adults, the subconscious state is gradually developed with a mean duration of five days with increasing drowsiness; confusion, dullness, and high fevers, and manifestation of seizures in estimated 15% of adults and 80% of children with severe malaria and frequently developed into the coma (Plewes *et al.*, 2018) and extramacular whitening.

Patients may recover full consciousness after a seizure; thus, transient postictal coma must be excluded. Multiple attacks are frequent, and up to 50% of unconscious children have subclinical convulsions or status epilepticus. Other clinical

manifestation includes; Ocular funduscopic findings which includes vessel color change, macular and white-centered retinal bleeding. The median time to coma recovery is roughly 24 hours in children and 48 hours in adults (Seydel *et al.,* 2015). There is the ability of retinal abnormalities to resolve with no residual visual deficit. Less than 1% of adults, and about but up to 12% of children in the quinine-therapy era could develop neurologic sequelae, including spastic paralysis, cortical blindness, aphasia, and cerebellar ataxia. Post malaria neurological syndrome is self-limiting; however, long exposure to neurological symptoms , including cognitive deficits and epilepsy, are reported among children (Plewes *et al.,* 2018). The quite a number of malaria patients are exposed to risk factors for developing acute kidney injury (AKI), which includes; volume depletion, hypoalbuminemia, concomitant bacterial sepsis, black water fever (BWF) co- morbidity, such as diabetes. Although oliguria clinically indicates decreased function with a pre-renal component, up to 80% of patients with malaria have nonoliguric AKI. Thus, the clinical symptoms if used will diagnosis anuric AKI but will under diagnose moderate AKI and delay diagnosis of the disease (Rocamora *et al.,* 2018). AKI complicating severe malaria are can be categorized into four; few severity criteria with pre-renal AKI that resolves with fluids, several severity criteria including AKI that resolves without renal replacement therapy(RRT), progressive AKI that resolves with antimalarial treatment and RRT, multiorgan dysfunction, often with anuric AKI and cerebral malaria, and those who die before or during RRT with hemodynamic shock and respiratory failure (Rudd *et al.,* 2018).

2.4 Diagnosis of Malaria Disease

Quality demonstration of malaria parasite is critical to proper malaria case management. The worldwide effect of malaria disease has propelled interest in

manufacturing successful testing strategies for resource-limited areas where malaria is a major public health concern in the community and developed countries, where malaria diagnostic expertise and equipment is often inadequate (Sharma *et al.*, 2017).

Malaria disease is a potential medical emergency and should to be treated urgently. Delays in diagnosis and treatment are the leading causes of death in many countries (Parpia *et al.,* 2016). Such delays can be due to many factors such as diagnosis can be confusing where malaria is no longer endemic for healthcare providers unfamiliar with the disease and clinicians may forget to consider malaria disease among some patients. Lack experience and technical skills from the laboratory personnel which can lead to failure to detect parasites when examining blood smears under a microscope.

Malaria can be diagnosed in the laboratory using various techniques, including microscopic diagnosis by use of stained thin and thick peripheral blood films (Jan *et al.*, 2018), concentration techniques, rapid diagnostic tests (mRDTs) and molecular diagnostic methods (Nijhuis *et al.*, 2018). This study evaluated the diagnostic performance of malaria rapid diagnostic tests using microscopy as the reference test method and determines the factors affecting the mRDTs.

2.4.1 Microscopy reference test method

Microscopic examination of stained blood films using Giemsa stains (Lavelle, 2018) is the gold standard for diagnosis of malaria. Microscopy is important for identification and detection of *Plasmodium* species when using thick films, whereas, thin blood films is useful for species' confirmation (Mukry *et al.*, 2017). In this method, the patient figure is sterilized with a 70% alcohol swab, leave for a few seconds to air dry, and then the side of the ring fingertip is pricked with a sterile lancet and a drop of blood is dropped on a glass slide. A blood spot on the slide is then spread in a circular motion

with the corner of another slide or spreader, taking care not to make too thick smear. A slide preparation template can be used to obtain a standard smear. The smear is allowed to air-dry. The blood spot is stained with working Giemsa solution (3%) for 30 minutes after it has air dried and rinsed by flooding the slide in buffered water for 3 minutes. The blood slide is then allowed to air-dry in a vertical position and examined under a microscope. The red cells lyse when a water-based stain is applied because the slides are not fixed.

A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45°, and spreading the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and fixed with absolute methanol, stained with working solution Giemsa (3%) for 30 minutes. The slide is rinsed by briefly dipping the slide in and out of a staining jar of buffered water. Allow the slide to air dry in a vertical position and examined under a microscope for the parasite's presence (Paul *et al.,* 2016).

Although the expert microscopist can detect up to 5parasites/µl, the average microscopist detects only 50-100 parasites/µl (Alnasser *et al.,* 2016). The low parasitemia level probably underestimated malaria percentage rate of infection, especially in low parasitemia individuals and asymptomatic malaria cases. The challenge associated with implementing and sustaining a level of skilled microscopy appropriate for clinical diagnosis, particularly in the field setting, has prompted the development of various malaria RDTs devices (Krampa *et al.*, 2017).

2.4.1.1 Advantages and disadvantages of microscopy

Microscopy is the most widely diagnostic tool used to demonstrate malaria at level 2 health facilities. In capable hands it is very sensitive for parasiteamia ≤50parasites/µL (0.001%) (Ezekiel, 2019) it gives species identification, parasitic stages and

parasiteamia level. However, quality of microscopy is difficult to implement and maintain. It is labor intensive and requires highly skilled personnel and constant regular quality assurance measures.

2.4.2 Rapid diagnostic test (RDT)

Malaria rapid diagnostic tests are immune-chromatographic tests, which are used to demonstrate the presence of malaria parasites in suspected malaria cases by detecting the presence of one or a combination of the following *Plasmodium* antigens. *Plasmodium* Histidine-rich protein (HRP)2 for *P. falciparum* or a 'pan-specific' aldolase to detect other species such as *P. vivax* or *Plasmodium* lactate dehydrogenase (pLDH) (Nyataya *et al.,* 2020).These are antibody base on the ability to capture circulating antigens from *Plasmodium* species, making them fast and reliable (Ifeorah *et al.,* 2017). There is numerous malaria RDTs commercially available, all of which detect malaria antigens or antibodies in the blood. Most mRDTs that detect *P. falciparum* are histidine rich protein-2(HRP2) based. Other tests detect the presence of parasite enzyme Lactate Dehydrogenase (pLDH), using either monoclonal antibodies that react with LDH of all species including *P. falciparum* (Pan or pLDH) or antibodies specific for *P. falciparum* LDH (Dayanand *et al.,* 2019).A blood sample from a patient need to be obtained using a lancet and standard sample collection devise is used to collect the blood sample and put in a sample well. Drop the reagent buffer solution on a test cassette, and interprets the results within 20 minutes (Abdalla *et al.,* 2019).

2.5 Types of malaria Rapid Diagnostic Tests kits

Malaria RDTs detect specific antigens (proteins) produced by malaria parasites that are present in the blood of infected individuals. Some RDTs detect a single species

(either *P. falciparum* or *P. vivax*), some detect multiple species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) and some further distinguish between *P. falciparum* and non-*P. falciparum* infection, or between specific species. Blood for the test is commonly obtained from a finger-prick and results are available within 15–30 minutes.

Histidine rich protein (Rock *et al.,* 1987) described how *P. falciparum*-infected RBC (IRBC) synthesize three histidine-rich proteins, HRP-1 (the knob-associated HRP), HRP-2 and HRP-3. HRP-1 (M_r 80,000 to 115,000) was identified in all knob-positive *P. falciparum* parasites but small amounts only were present in Gambian isolates and in several culture-adapted strains. HRP-2 (M_r 60,000 to 105,000) was identified in all *P. falciparum* parasites regardless of the knob phenotype and was recovered from culture supernatants as a secreted water-soluble protein. HRP-2 was shown to be a surface-exposed protein complex of several close bands. HRP-3 (M_r 40,000 to 55,000) was present at the lowest abundance compared to HRP-1 and HRP-2. Neither HRP-1 nor HRP-2 was found in a range of other knob-positive and negative strains of non-*P. falciparum* malaria.HRP-2 is a water-soluble protein produced by asexual stages and young gametocytes of *P. falciparum* (Obeagu *et al.,* 2018) .It is expressed on the RBC membrane surface, and because of its abundance in *P. falciparum,* it was the first antigen to be used to develop an RDT for its detection.

Plasmodium lactate dehydrogenase pLDH an enzyme found in the glycolytic pathway of the malaria parasite is produced by sexual and asexual stages of the parasite (Kumar *et al.,* 2020). Different isomers of pLDH for each of the four *Plasmodium* spp. infecting humans exist, and their detection constitutes a second approach to mRDTs development. Several other enzymes of the malaria parasite glycolytic pathway,

notably aldolase (Maran *et al.,* 2021) have been suggested as target antigens for RDT for species other than *P. falciparum.*

Though there are variations among the more than 200 malaria RDT products on the market, the principles of the tests are similar. Immunochromatography relies on the migration of liquid across the surface of a nitrocellulose membrane. Immunochromatographic tests are based on the capture of parasite antigen from peripheral blood using monoclonal antibodies prepared against a malaria antigen target and conjugated to either a liposome containing selenium dye or gold particles in a mobile phase. A second or third capture monoclonal antibody applied to a strip of nitrocellulose acts as the immobile phase. The migration of the antigen-antibody complex in the mobile phase along the strip enables the labeled antigen to be captured by the monoclonal antibody of the immobile phase, thus producing a visible colored line. Incorporation of a labeled goat anti-mouse antibody capture ensures that the system is controlled for migration (Willie, 2018) (Fig 2.1).

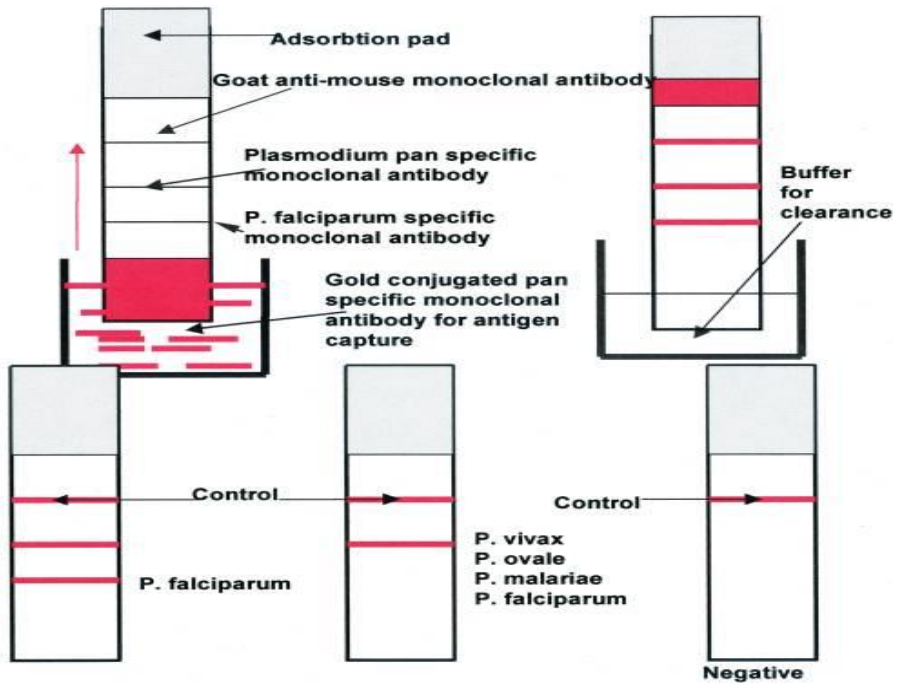


Figure 2.1 Principle of immunochromatographic RDT for malaria

2.5.1 Advantages and disadvantages of mRDTs

Systematic reviews have shown that mRDTs give high-quality results , reliable and are cost-effective (Recht *et al.,* 2018). The mRDTs procedure is simple and do not require high levels of technical expertise enhance the ability to be used by a wide range of personnel, including community health volunteers, which may constantly reduce the miss use of antimalarial drugs. Studies have shown rapid test recording the highest sensitivity (100%) and accuracy (100%) and sensitivity 89.0% and accuracy 91.5% respectively (Grigg *et al.,* 2014; Jang *et al.,* 2013; Vásquez *et al.,* 2018; Wanja *et al.,* 2016b). However, performance characteristics decreases as parasitemia level decrease to less than 500 parasites/µl (Venkatesh *et al.,* 2016). HRP2 RDTs have disadvantages as their performance has been shown to be affected by product quality and parasite-related factors such as *pfhrp2/3* gene deletion, non-*P*. *falciparum* species and prozone effects that leads to false negative mRDTs (Gendrot *et al.,* 2019). The presence of *pfhrp2* and *pfhrp3* gene deletions in *P*. *falciparum* parasite populations has been reported in Uganda (Bosco *et al.,* 2020) and other malaria endemic countries in sub-Saharan Africa (Agaba *et al.,* 2019). HRP2 RDTs may not detect other species of *Plasmodium* parasite this can cause false-negative RDT results, leading to a reduction in the effectiveness of rapid diagnostic tests (Gatton *et al.,* 2020). Evidence of the possible contributions of parasite gene deletions, non-*P*. *falciparum* species and low-density infections to false-negative HRP2 RDT results in Seme are limited. As Kisumu advances towards malaria elimination, it is important to ensure that all malaria infections are detected by effective diagnostic tools and treated promptly to enhance case management and surveillance-based interventions.

2.6 Evaluating the performance of mRDTs

The development of RDTs has been a significant step forward in attempts of parasitological diagnosis of malaria. These tests extend malaria diagnosis to populations with no access to excellent microscopy services. Malaria RDTs make this possible because of their: ease of use, lower training requirements, lack of provisions for electricity, or expensive equipment (Beisel *et al.,* 2016). Therefore, the available RDTs must give accurate results.

The performance of mRDT can be determined using microscopy, which is the gold standard. It is essential to determine the quality indicators to evaluate how useful the test can detect a disease or characteristic in the given population. It is essential to know how the test is likely to be sensitive, its specificity, and how likely someone with a positive test result is to have the character (positive predictive value). Besides, it's possible for someone with a negative test result not to have the attribute (negative predictive value).

A similar study done by Wanja revealed the following results, the sensitivity and specificity of RDTs to detect *P. falciparum* (HRP2-band) against microscopy was relatively similar across all the eight malaria RDTs tested. The sensitivity was shown to be >90 % while the specificity was shown to be >73 % when compared to microscopy. The SD Bioline 05FK50 kit had the highest sensitivity of 94.8 %, while First Response I16FRC kit had the lowest sensitivity of 90.4 %. The specificity of all the eight malaria kits was >73 % when compared to microscopy. The Binax NOW 665-025 kit had the highest specificity of 79.3 %.While SD Bioline 05FK50 kit had the lowest specificity of 73.3 % (Wanja *et al.,* 2016a).

The study gap addressed in this particular study was the determination of the accuracy of mRDTs used for diagnosis of malaria using microscopy as the reference method, in Seme Sub-County. The various factors affecting the performance of mRDTs and the quantitative detective limit of malaria parasite density that can be detected by the multiple mRDTs were also addressed. The mRDTs performance evaluation was carried out using protocol adopted from the WHO product testing program, which is part of the WHO-FIND malaria RDT evaluation program (Round 3 2010-2011).

2.7 Sensitivity, Specificity, and predictive values of Rapid diagnostic tests

Microscopy is considered the gold standard for the diagnosis of malaria (Berzosa *et al.,* 2018). Hence the use of microscopy to determine the specificity, sensitivity and predictive values of mRDTs. A research done in Cameroon revealed that the prevalence of malaria by microscopy and RDT was 31% and 45%, respectively. In the study 23% of individuals had sub-microscopic infections .The results of the study was as follows; The sensitivity of microscopy-57%, mRDT-78%, and clinical diagnosis-100%; and the specificity was as follows; 99%, 94%, and 17% respectively; the positive predictive values for microscopy was 99%,mRDTs- 94%, and clinical diagnosis -59%; whereas the negative predictive values were 66% for microscopy, 78% for RDTs, and 100% for clinical (Mfuh *et al.,* 2019).

Studies have shown decreasing sensitivity at low parasiteamia. A survey conducted in four (non-endemic areas) in patients with symptoms suggestive of malaria recorded 100% sensitivity for $\geq$ 500 *P. falciparum* parasites/µl, which reduces to $\leq$ 73% at lower parasite density (<500 parasites/µl). A study conducted in Port-Harcourt, Nigeria, Madagascar, and mount Cameroon Region (Obeagu *et al.,* 2018). In the study pLDH, detecting Pan-specific optimal was used. The sensitivity found was related to the

species of the *Plasmodium* parasite present in the blood sample. In the study done in London, recorded Performance of OptiMAL malaria antigen capture, 95.3% sensitivity was or *Plasmodium falciparum, Plasmodium vivax* (96%), *Plasmodium ovale* (57%), and *Plasmodium malaria* (47%). The research found the last two to be relatively low (Haider *et al.,* 2020). Previous works elucidated the dependence of sensitivity on parasite species (Baum *et al.,* 2015; Slater *et al.,* 2019) . The relationship of sensitivity could be due to defective mRDTs kits and or change in predominant *Plasmodium* species studies in Enugu and Ethiopia showed low sensitivity of a *P.falciparum*. RDT and para screen RDT irrespective of parasite density or species variability (Shago *et al.,* 2020). Calculation of quality indicators (table 2.1).

Table 2.1 Calculation of quality indicators.

		Disease number	Non-disease number	Total number
TEST RESULTS	Positive number	A True positive	B False-positive	T Test positive
	Negative number	C False-negative	C True negative	T Test negative
		T Disease	T Non-disease	Total

2.8 Estimation of the quantitative limit of parasite density cut off points

Microscopy is the gold standard for identifying malaria parasites in blood (Das *et al.,* 2015). Thick films are performed to detect parasites and measure parasite density (quantification) and can be used to monitor response to treatment. Parasites are quantified by counting ring forms (trophozoites) against white blood cells. It is recommended in routine practice that parasite count be performed against 200 or 500 WBCs (Bosoka *et al.,* 2017). If, after counting 200 WBCs, 100 or more parasites are

found, record the results in terms of the number of parasites /200 WBC. If < 100 parasites are found after counting 200 WBCs, parasite quantification should be continued until 500 WBCs are counted. This method of quantification is essential in low and moderate parasitemia. Thin films are prepared for parasite species identification and quantification also. The percentage of parasitized red cells is determined by counting the numbers of red cells and parasitized red cells. This method of quantification is useful in high parasitemia.

Process of counting malaria parasites ensures that an adequate number of microscopic fields are examined (preferably 100 areas or more in a thick blood film). Considering the 100 areas is essential even if the blood film is positive to detect other malaria parasites (mixed infection) or any other blood parasites present. To quantify malaria parasites against Red blood cells, count the infected red blood cells among 500-2,000 RBCs on the thin smear, and express the results as % parasitemia.

% parasitemia = (infected RBCs/total RBCs) × 100 (WHO, 2015)

If the parasiteamia is high (for example> 10%) examine 500 RBCs; if it is low (for example <1% examine 2,000 RBCs (or more); count asexual blood-stage parasites and gametocytes separately. Only the former is clinically important, and gametocytes of *P. falciparum* can persist after the elimination of asexual stages by drug treatment.

To quantify malaria parasites against white blood cells: on the thick blood film, tally the parasites against WBCs, until you have counted 500 parasites or 1,000 WBCs, whichever comes first; express the results as parasites per microliter of blood, using the WBC count if known, or otherwise assuming 8,000 WBCs per microliter blood. Parasites/microliter blood= (parasites/WBCs) × WBC count per microliter <or

8,000>Results in % parasitized RBCs and parasites per microliter blood can be converted if the WBC and RBC counts are known, or otherwise (less desirably), by assuming 8,000 WBCs and 4,000,000 RBCs per microliter blood (Imwong *et al.*, 2016).

2.9 Factors affecting the diagnostic performance of malaria RDTs

Various factors could affect the diagnostic accuracy of mRDT. These can be broadly categorized into test device-related factors (quality control/ assurance, storage, transportation) handling environmental conditions (Obeagu *et al.*, 2018). Preparation and interpretation of the results, volume of blood and buffer, age and storage of blood sample, antigen, density, and species. Others include host-related factors such as the treatment history and effectiveness of treatment. Interpretation of mRDT results should consider the clinical history of antimalarial treatment because of delayed parasite clearance (>1 month) for the HRP-2 antigen (Dalrymple *et al.*, 2018b).

Extreme hot temperatures above 30°C is unsuitable for storing mRDTs by the WHO (Tamiru *et al.*, 2015). Extreme temperatures can interfere with the overall performance of the mRDTs, and effective temperature-monitoring is essential in all laboratories and climates. Air-conditioning systems or similar cooling equipment should be considered in those facility stores that exceed the WHO recommended threshold. Malaria RDT should be stored in a centralized store as long as possible, and keen monitoring of temperatures during transportation and storage at the health facilities to minimize degradation. The use of positive control wells and temperature monitors should be considered in facilities to assure the quality of the mRDTs and build the confidence of the users on mRDTs (Bell *et al.,* 2017). There is little information on the epidemiological factors that influence the specificity of HRP2-and pLDH based tests.

In this study, the researcher determined the factors affecting diagnostic performance of mRDTs.

Although the procedure of using mRDTs may seem simple, the diagnostic efficacy is subject to many factors, e.g., the different species of malaria parasites, erythrocytic schizogony, endemicity of different species, the interrelation between levels of transmission, population movement, parasitemia, immunity, and signs and symptoms (Bosco *et al.,* 2020). Other factors include drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasiteamia level, and sequestration of the *Plasmodium* parasites in the deeper tissues or organs, and the use of chemoprophylaxis or even presumptive treatment based on clinical diagnosis, can all influence the identification and interpretation of malaria parasiteamia in a diagnostic test (Ayogu, 2017).

2.10 Effects of inaccurate diagnosis of malaria

The misdiagnosis of malaria disease can lead to medical, social, and economic effects (Soofi *et al.,* 2019). Therapeutic effects of misdiagnosis at the individual level include inappropriate treatment resulting in a prolonged illness (Boyce *et al.,* 2017). Social effects could consist of a loss of faith in health care services and delayed care-seeking. Economic effects could include loss of earnings or increased expenditure on transport, drugs, and consultations.

The effects of false-positive results of malaria could result in higher mortality from diseases other than malaria (Oleribe *et al.,* 2017). Reyburn and colleagues demonstrated in Tanzania that clinical diagnosis yielded more false positives than diagnosis using microscopy (Ravenhall *et al.,* 2018; Reyburn *et al.,* 2007). False-positive malaria test results can lead to the overuse of antimalarial drugs and may even

prevent other diagnoses and treatments of other diseases. On the other hand, false-negative malaria results could result in a lack of treatment of patients and lead to malaria-related complications and even death (Recht *et al.*, 2017). A study done in Eastern Sudan indicates that mRDTs have poor performance in detecting placental *P. falciparum* malaria. Malaria RDT had low sensitivity (17.4%) and specificity (81.7) for diagnosing placental malaria when compared with PCR as the gold standard (Kashif *et al.*, 2013). This low sensitivity can result to still birth, threaten abortion and even death of the pregnant women. Therefore, ensuring the diagnostic accuracy of malaria RDTs is essential to prevent the unnecessary and avoidable consequences of over-or under-diagnosis of malaria. The evaluation of the diagnostic performance of RDTs in Seme Sub County and the factors affecting their performance presented in this study would inform the policymakers on the right diagnostic tool to use.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Seme Sub County, situated approximately 35km West of Kisumu city, along Kisumu Bondo highway, (Lat 0.103661/long 34.518190). Kombewa County hospital is the central hub. The study facilities included Miranga, which is 9.4km from the central hub, Ratta 16.4km, and Manyuada Sub County hospital 10km. Seme Sub County has a total population of 98,805 with an area of 190.20km^{2}and 25 health facilities. The study facilities had different catchment population as indicated in the brackets, Miranga (9,864), Manyuada (10,011), and Ratta (14,059) as obtained from Kenya Health Information System aggregate 2019. Kisumu West, part of Kisumu central Sub Counties and Rarieda Sub County in Siaya County, borders Seme. Patients from the neighboring Sub Counties also seek care at the Hospitals within Seme Sub County. The Hospitals serve the majority of the malaria-infected patient and it is among the top ten diseases causing morbidity and mortality within the region as seen Kenya Health information System dashboard.

The hospitals are along the lake region hence the high incidence rate of malaria infection. There are frequent power outbreak and no backup systems in place in most of the hospitals. Road infrastructure is good, though problematic during rainy seasons. Economic activities within the area are majorly fishing because of the lake, beaches, and surrounding Islands, and small-scale farming is taking place. However, the majority are also engaged in small-scale businesses to earn a living at the locally available market centers (Appendix I).

3.2 Study Design

Across-sectional analytical design was used. The mRDTs kits evaluated were ParaScreen rapid test for malaria pan/*Pf* (Lot 101343) manufactured by Zephyr Biomedicals in India and SD ^{BIOLINE} malaria Ag *P.f* (lot 05FK50) manufactured by Abbot standard diagnostic Inc. in Korea. Whole blood sample panels (223) were subjected to the two different RDTs each and the reference method "microscopy". Data was collected through laboratory analytical procedures, observation and by the use of questionnaires for objective three.

3.3 Study objects

The study objects, were Histidine rich protein2 (HRP2) rapid diagnostic test kits and parasite lactate dehydrogenase combined with HRP2 based rapid diagnostic test (HRP2/pLDH). The accessible population were the three health facilities (Ratta, Miranga and Manyuada) within Seme Sub County.

3.3.1 Inclusion criteria

The study included HRP2 (SD ^{BIOLINE} malaria Ag *Pf* -05FK50) of batch number 05CDF08-3A manufactured by Abbot standard Diagnostic Inc. in Korea and HRP2/pLDH RDTs (ParaScreen rapid test for malaria pan/*Pf)*batch 101343 manufactured by Zephyr Biomedicals in India. The mRDTs were of good condition, free from physical damage and were within the self-life given by the manufacturer. The sample panels must be from the patients send to the laboratory suspected to have malaria for the confirmatory parasitological test.

3.3.2 Exclusion criteria

The study excluded all the expired and damaged rapid diagnostic tests kits. It also excluded other types of rapid diagnostic test kits. Sample from other patients who were not suspected to have malaria and therefore not sent for confirmatory parasitological test were excluded and not sampled to make part of the sample panel.

3.4 Study Variables

The study had independent, intervening and dependent variables that guided the study to meet the objectives.

3.4.1 Dependent variables

The dependent variable in this case would be the quality performance of RDTs. This is because the quality of rapid diagnostic test depends on environmental conditions and inbuilt test characteristics. Such factors include the sensitivity, specificity and predictive value and environment factors such as storage conditions, staff training, and use of quality control wells, standard operating procedures and malaria diagnostic guidelines.

3.4.2 Independent variable

In this study, the independent variables were the sensitivity, specificity, predictive values. It also included parasiteamia level, level of magnification and factors affecting the performance of RDTs such as storage, staff training, quality control measures and use of standard operating procedures and malaria diagnostic guidelines. All these independent variables lead to the quality performance of the rapid diagnostic test.

3.5 Sampling design

Purposive sampling design to select the health facilities was used. The facilities were proportionally divided according to the target objects expected to be evaluated. The rapid diagnostic test kits were randomly sampled. Convenience sampling was also used to administer the questionnaires to the health care workers.

3.6 Sample Size Determination

The Cochran's formula was used (Israel, 1992) to determine the sample size which gave us the number of mRDTs to be evaluated. The three health facilities from the 25 were purposively selected. The catchment population per facility were as follows (Miranga = 9,864, Ratta =14,059 and Manyuada =10,011). Which were used to determine the sample size giving us the number of mRDTs kits to be evaluated in the study.

Cochran's formula is

$$\underline{n_0 = Z^2 Pq}$$

$$e^2$$

The initials represent the following:

n= sample size (where the population is more than 10,000)

e is the desired level of precision (i.e., the margin of error),

p is the proportion of the population, which has the attribute in question, (This is the prevalence of malaria (28%) according to a study done 2015 in Maseno, Kisumu West Sub County (Jenkins., *et al*. 2015). The study adopted the prevalence rate because

Seme and Kisumu west Sub County initially was Kisumu rural District and regarded as one; hence the study also covered Seme Sub County by then.

Q Is 1 – p.
The z value is found in the z table.

$$N_o = \frac{(1.96)^2(0.28)\,(0.72)^2}{(0.05)^2}$$

$$\frac{3.8416 \times 0.28 \times 0.5184}{0.0025}$$

$n_o = 223$

Sample size = 223

This form sample panels used to evaluate mRDTs using microscopy as the reference method. The target health population density was used to obtain the study hospitals' sample size by getting the percentage population of each hospital from the total study population (33,931) obtained from KHIS aggregate population data. Convenience sampling was used to select Miranga, Ratta and Manyuada health facilities and the health care workers to be interviewed were purposively selected. Proportional random sampling was done in each health facility to achieve the desired sample size.

Table 3.1 Distributions of the study objects (mRDTs test kits) in the health facilities

Health facility	Catchment population	Sampled mRDTs
Miranga	9864	$\frac{9864\ x\ 223}{33931} = 65$
Ratta	14059	$\frac{14059\ x\ 223}{33931} = 92$
Manyuada	10011	$\frac{10011\ x\ 223}{33931} = 66$
Total	33931	223

Table 3.2 Distribution of the study objects as per the diagnostic method

Diagnostic method	Reference method (microscopy)	HRP2 RDTs	HRP2/pLDH RDTs
Sample size	223 Ratta (65) Miranga(92) Manyuada(66)	223 Ratta (65) Miranga(92) Manyuada(66)	223 Ratta (65) Miranga(92) Manyuada(66)

3.7 Data collection process

Data collection instruments used included laboratory procedures, questionnaires and observations.

3.7.1 Field assistants' training

The laboratory diagnosis team was composed of six medical laboratory officers with a minimum diploma in medical laboratory sciences training and previous clinical laboratory unit experience. The six were divided into two categories. Three medical laboratory officers were tasked with sample collection, performing malaria rapid diagnostic tests, and preparing and staining the thick and thin smears. The three had pre-training on phlebotomy and orientation on safe phlebotomy, malaria microscopy, and malaria case management training. On job training and orientation was done according to WHO standard operating procedures used in the study. The second category of the laboratory officers was responsible for microscopy slide reading. This group of officers were well trained in malaria microscopy and passed a competency assessment. All the laboratory officers were supplied with operational procedures manual regarding laboratory diagnosis procedures.

3.7.2 Collection of specimen and quality assurance

Patients' whole blood samples were used to evaluate the diagnostic tests against the reference method. Using standard phlebotomy procedure, 2mL of venous blood was drawn and dispensed into ethylenediaminetetraacetic acid anticoagulant (EDTA) tubes by qualified medical laboratory officer working in the health facility. The sample was then taken to the laboratory and used for the Rapid Diagnostic Test and microscopy testing. All collection sites were assessed for competency at conducting routine and reference malaria diagnosis. Personnel selection was based on experience in routine, reference and study diagnostic methods. Internal and external quality assurance programme was in place.

3.7.3 Testing for malaria disease using RDTs

Rapid Diagnostic test was carried out as routinely done without any special attention given to the samples by medical laboratory officer working in the health facilities laboratory. All rapid diagnostic tests were done using SD^{BIOLINE} malaria antigen *Pf*. Batch number 05CDEF08-3Amanufactured by Abbot standard diagnostic Inc., Korea and ParaScreen rapid test for malaria pan/ Pf, manufactured by Zephyr Biomedicals in India.

In brief, all kit components and specimen were assembled together. Using a 5 μl disposable capillary pipette, whole blood was drawn and transferred into the round sample well. Four (4) drops of assay diluent was added into the square assay diluent well holding the diluent bottle vertically. Reading of test was done in 15 minutes for SD ^{BIOLINE} and 20 minutes for ParaScreen. The presence of one colour band (“C” Control line) within the result window was interpreted as negative results. The presence of two colour bands (“T” Test line and “C” Control line) within the result window was interpreted as positive results. In the event where the control line fails to

appear within the results window, the test was invalid (repeat the test). Report the results as “mRDT Negative” or “mRDT Positive” or “mRDT Invalid” (fig 3.1).

POSITIVE

NEGATIVE

INVALID

INVALID

Figure 3.1 Interpretation of malaria rapid diagnostic test results

3.7.4 Testing for malaria using microscopy

An amount of 6 μL and 2 μL of the blood sample was pipetted for the preparation of thick and thin blood films, respectively. Thin film was fixed with methanol for 5 minutes and both thin and thick film were stained with 3 % Giemsa for 30 minutes. Stained slides were left to air dry before examination using a 100x objective oil immersion light microscope. The smears were independently read by two

microscopists who were blinded to the results of the mRDT as well as the diagnosis made by the clinicians and between each other.

3.7.4.1 Quantification of malaria parasite density

Examination of the slides were done and if malaria parasites were present, the asexual forms were counted without sexual (gametocyte) forms, in mixed infections, all asexual parasites were counted together. Look for afield with a good number of white cells and parasites observed together starting at the topmost left part of the film and start counting. Two tally counters were used one for the WBCs and the other for parasites. Counting was done for all the parasites and white cells in one field, then movement was done to the next field following a systemic pattern. The following rules were followed during the counting, if ≥100 parasites in 200 white cells have been counted, counting was stopped and results recorded as the number of parasites per 200 white cells. If ≤99 parasites in 500 white cells were counted, counting was stopped and results recorded as the number of parasites per 500 white cells. All the parasites and WBCs were counted in the last field, even if the white cell count exceeds 200 or 500. The actual numbers of parasites and white cells counted were recorded on an appropriate worksheet. Calculation of the parasite density based on the patient's actual white cell count was done as follows.

$$\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted x 8000 white cells/}\mu\text{L}}{\text{No. of white cells counted}}$$

$$\text{Sensitivity} = \frac{\text{Number of true positives (TP or A) using the reference method}}{\text{[Number of true positives (TP or A)] + [Number of false negatives (FN or C)]}}$$

$$\text{Specificity} = \frac{\text{Number of true negative (TN or C) using the reference method}}{\text{[Number of true negatives (TN or C)] + [Number of false positives (FN or C)}}$$

$$\text{Predictive Value} = \text{True } \frac{\text{Positive (A)} \times 100}{}$$

True Positive (A) + False negative (B)

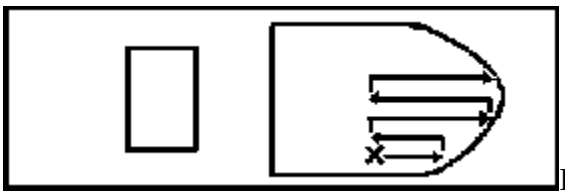
Negative Predictive Value = True Negative (D) × 100

True Negative (D) + False Negative(C)

False positive rate =False positive (FP)

False-positive (FP) +True negative (TN)

The results were recorded and reported after microscopy examination. All the malaria indicators were reported during smear examination. Record the results in the laboratory results tracking log. The date, time of examination and parasite species, stages and count. The reporting was uniformly done. For example, *P. vivax* trophozoites seen. *P. falciparum* trophozoites seen; count, 42 000 parasites/µL. · *P. falciparum* gametocytes seen. No malaria parasites were seen (Figure 3.2).


Examination of a thin blood film

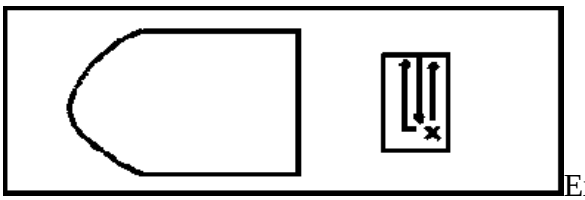

Examination of a thick blood film

Figure 3.2 Routine examination of blood films for malaria parasites

3.7.5 Factors affecting the performance of mRDTs

For the purpose of this study, in depth interviews and observation were used to obtain the factors affecting the performance of mRDTs. Semi-structured questionnaires were used to interview and observe the health care workers to assess these factors. The health care workers were purposefully selected and they included clinicians, medical laboratory officers, nurses and community health volunteers. The interviews were transcribed, ordered and coded in matrixes using the key categories of commodity

storage, stock availability and monitoring, staff training, adherence to standard operative procedures and end user experiences. (Appendix II).

3.7.6 Reliability and validity

Calibrated pipette was used to obtain a standard amount of blood. To achieve a good quality of staining and standardization of blood film examination and reporting, the blood spreader was used to spread the blood film evenly over a specified area of the slide using a slide preparation template. Each slide was subjected to preliminary screening using low power objectives, followed by examining at least 100 microscopic fields using high power objectives×100. Two microscopic experts examined slides, and a third expert reviewed slides with discrepant results.

For malaria RDTs, the C band's presence was an indicator of the test's validity. The standard operating procedures (SOPs) were followed before the start of the process using the manufacturers' instructions available on the reagent insert; the training done included proper handling of the RDTs, collection of blood with the pipette for testing on the RDTs, use of the reagent buffer for RDT and the reading and interpretation of the test from the RDTs.

3.7.7 Quality assurance

The quality assurance officer reviewed methods for preparing both the thin and thick blood films, staining, species identification, and parasite quantification guideline as per the WHO 2016 guideline. Uniformity in classifying test results as either "positive" or "negative" as described in the manufacturers' instructions for the mRDTs was reviewed appropriately. A blinded test sample was used as part of the quality assurance process. Known positive and negative control slides were prepared and kept for quality

control. Known positive and negative blood samples from the previous day's work was used to internally control the mRDTs.

3.7.8 Handling indeterminate/Invalid results

Indeterminate results were recorded as such. The test was repeated using a new test kit device and the same whole blood sample. The results for the repeat tests were documented on the result trucking log.

3.8 Standard operating procedure for performing reproducibility testing

Two microscopic experts independently read and recorded the results of each testing. In case the results from the two experts were inconclusive, a third microscopic expert was brought on board to act as a tire breaker. Two similar results were picked to be the right one.

3.9 Pilot phase

Tests were performed with positive and negative whole blood samples, under supervision. The diagnostic tests were piloted in parallel with the reference method. The tests were blinded to the test results. The results were read and when results were invalid the test was repeated with new devices. The researcher and the assistants proceeded with the evaluation only when they were confident about each component of the testing procedure.

3.10 Data management and analysis

Primary data was documented in the results tracking log and keyed in a password-protected Microsoft Office Excel 2010. Data completeness and consistency was checked and data queries resolved after data entry. Analysis of data was done using STATA software version 15.

Cohen's Kappa coefficient was used to observe the agreement among the different diagnostic tests being evaluated and McNemar was used to check the discordance between the diagnostic tests. Standard WHO procedures were used to calculate sensitivity, specificity and predictive values.

The likelihood ratio was used to determine the quantitative limit of parasite density cut off points detected by the different mRDTs compared to microscopy and receiver operating characteristic (ROC) was used to get the statistical significance of the quantitative limit of parasite density cut off.

Descriptive data analysis was used to describe factors affecting the performance of mRDTs and presentation done in frequencies, proportions, percentages, dispersion measures, and central tendencies in tables.

3.11 Logistical and Ethical Considerations

Ethical approval was sought from the scientific and ethical approvals MMUST IREC, NACOSTI, and permission obtained from the Sub County Health Management team. There was respect to the autonomy of all participants and participants with diminished autonomy were entitled to additional protection. They were allowed to go through the process as fast as possible to minimize their hospital stay. Participation was voluntary from respondents and the right to refuse or drop from the study at any phase if they wish to. Meaning, non-prejudicial treatment of those who decline to participate or withdraw from the study after agreeing to participate was accepted. Participation was based on informed written consent and permission from the caretakers for the minors based on assent (Appendix III and IV). Sufficient information about the purpose and objectives of the study was provided to the participants. To make participants give assurances about taking part to allow individuals to understand the implications of

participation, agree on informed consent, and willingly decide whether or not to do so, without any pressure or persuasion.

Counselling was done to the participants before signing the informed consent form. Use of offensive, discriminatory, or any other unacceptable language to formulate the questionnaires was avoided. Training of the research assistants on good clinical practice (GCP) and ethical issues was done to enable the study to achieve the ethical considerations' expectations. Unique identifier or codes to maintain privacy and anonymity were used. To ensure adequate study data confidentiality at all levels; the documents were kept in a lockable cabinet and have a password protected excel sheet for data storage. The study staff accessing the information signed a statement of intent (Appendix V). The study objectives were clearly explained to the participants, and no deception or exaggeration about the study was allowed.

A trained phlebotomist did blood sample collection to avoid harm, and the participants were treated with respect for dignity. There was no transport of samples from one study area to another. The findings and feedback was given with honesty and transparency and there was no provision for the participants' compensation. There was no conflict of interest in this study. The blood sample disposal was done after use as per the normal sample disposal protocol for clinical samples, and nobody was allowed access to them, apart from the study staffs. The study did not allow any misleading information as well as the representation of primary data findings. There were fair and non-discriminatory participation and participants' selection such that any risks and benefits were to be equitably shared. There was respect to cultural and other forms of human diversity before, during, and after the study's participants.

CHAPTER FOUR

4.0 RESULTS

4.1 Sensitivity, Specificity, and Predictive Values of HRP2 based mRDTs and HRP2/pLDH based mRDTs

The HRP2 mRDT and HRP2/pLDH mRDT had similar level of sensitivity at 94.4% (95 % CI = 0.87 – 0.98). The specificity of the two mRDTs kits against microscopy was found to be 85.7 % (95 % CI = 0.79 - 0.91) and 85.0 % (95 % CI = 0.78 - 0.90) for HRP2 mRDTs and HRP2/pLDH mRDTs respectively. HRP2 mRDT had a positive predictive value of 81.0 % (95 % CI = 0.72 - 0.87) and HRP2/pLDH mRDT had a positive predictive value of 80.2 % (95 % CI = 0.71 – 0.87).The study found the negative predictive value for HRP2 mRDT to be 96.0 % (95 % CI = 0.91 – 0.98), and that of HRP2/pLDH mRDT to be 96.0 % (95 %CI = 0.91 – 0.98).

The McNemar χ^2 two-sided exact test applied in Table 4.1 shows that there is significant discordance between the proportions of positive results by HRP2 mRDTs and positive results by microscopy (P = .004). On the other hand, under pLDH-mRDT, the McNemar χ^2 two-sided exact test has a P-value that is less than 5% (P = .002). This shows that there is a statistical significant discordance of the proportion of positive results among diseased (sensitivity) for the pLDH mRDT test against microscopy test.

Cohen's Kappa was also run to determine if there was a significant agreement between microscopy test and mRDTs of malaria on whether respondents test positive or negative. There was a good agreement between microscopy and HRP2 mRDT (k = 0.78, 95% CI = 0.69-0.86, P < .001) and also there was a good agreement between microscopy and pLDH mRDT (k = 0.77, 95% CI = 0.69-0.84, P < .001)

Table 4.1 Sensitivity, Specificity, and Predictive Values of mRDTs using microscopy as a gold standard and their level of agreement

	HRP2-mRDTs	pLDH mRDTs
Sensitivity (95%CI)	94.4 (0.87 - 0.98)	94.4 (0.87 - 0.98)
Specificity (95%CI)	85.7 (0.79 - 0.91)	85.0 (0.78 - 0.90)
Positive predictive value (95%CI)	81.0 (0.72 - 0.87)	80.2 (0.71 - 0.87)
Negative predictive value (95%CI)	96.0 (0.91 - 0.98)	96.0 (0.91 - 0.98)
MCNemar's χ^2	***P* = .004**	***P* = .002**
Kappa value (95%CI)	0.8 (0.69 – 0.86)	0.8 (0.69 – 0.84)
Kappa	***P* < .001**	***P* < .001**

CI= Confidence interval; ***P***=P-value

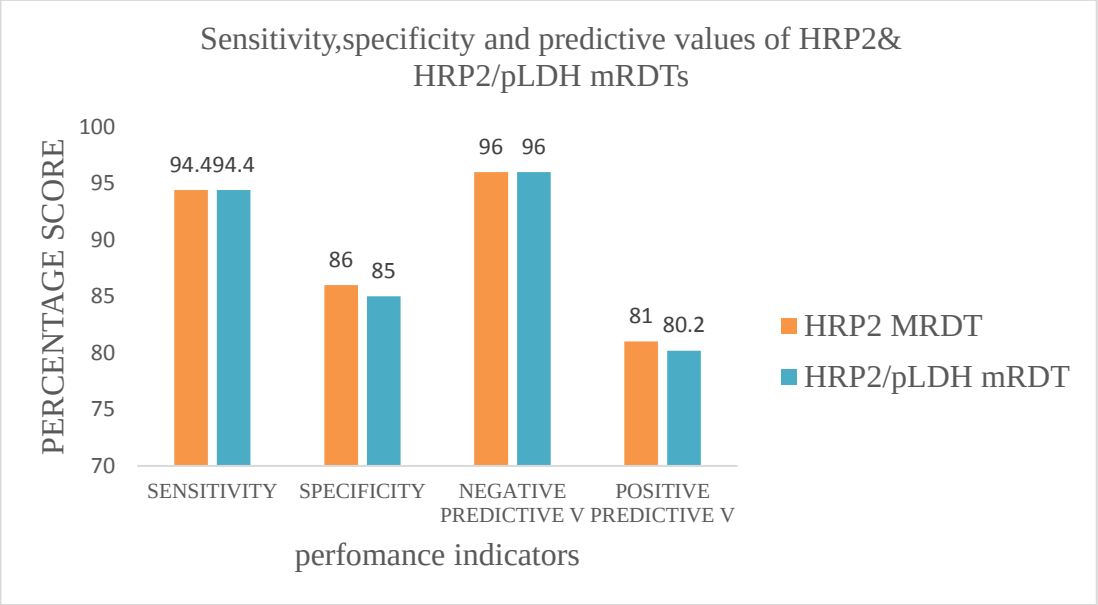


Figure 4.1 sensitivity, specificity and predictive values of HRP2 and pLDH mRD

4.2 Quantitative limit of parasite density cut off points detected by the different RDTs

The performances of mRDTs were considered at cut-off point criteria of 80, 100, 500, 1000, 50000, 10000, 50000, and 100000 parasite/µl of blood. The sensitivity of both HRP2 mRDT, and HRP2/pLDH mRDT was high at malaria parasite density of 80

parasite/µl to 1000 parasite/µl as shown in Tables 4.2 and 4.3 the specificity was high at >1000000 parasite/µl of blood. The study also found a positive likelihood ratio (LR+) to be high at parasite density cut-off point of 80 and 100 parasite/µl for both HRP2 and HRP2/pLDH mRDTs (LR+> =1). As the density cut-off point is increased from 500 to 100000 parasite/µl, the probability that a person without malaria testing positive decreases. The study also found that quantitative limit of parasite density cut off points to be at 80 parasites/µl of blood.

Table 4.2 Diagnostic performance of the HRP2 RDTs kit compared to microscopy exploring parasite density cut-off points

Correctly Cut point	Sensitivity	Specificity	Classified	Positive Likelihood Ratio (LR+)	Negative Likelihood Ratio (LR-)
>= 80	100.0 %	0.0 %	45.7 %	1	
>= 100	100.0 %	0.8 %	46.1 %	1.0081	0
>= 500	98.1 %	0.8 %	45.2 %	0.9889	2.3809
>= 1000	80.0 %	0.8 %	37.0 %	0.8065	24.9999
>= 5000	51.4 %	2.4 %	24.8 %	0.5269	20.2381
>= 10000	50.5 %	2.4 %	24.4 %	0.5172	20.6349
>= 50000	31.4 %	4.0 %	16.5 %	0.3274	17.1428
>= 100000	23.8 %	4.0 %	13.0 %	0.248	19.0476
> 100000	0.0 %	100.0 %	54.4 %		1

LR+ (Positive likelihood Ratio) (**LR-** Likelihood Ratio)

Table 4.3 Diagnostic performance of the HRP2/pLDH RDTs kit compared to microscopy exploring parasite density cut-off points

Correctly Cut point	Sensitivity	Specificity	Classified	Positive Likelihood Ratio (LR+)	Negative Likelihood Ratio (LR-)
>= 80	100.0 %	0.0 %	46.09%	1	
>= 100	100.0 %	0.8 %	46.52%	1.0081	0
>= 500	98.1 %	0.8 %	45.65%	0.9891	2.3396
>= 1000	80.2 %	0.8 %	37.39%	0.8084	24.5661
>= 5000	51.9 %	2.4 %	25.22%	0.5317	19.8868
>= 10000	50.9 %	2.4 %	24.78%	0.5221	20.2767
>= 50000	32.1 %	4.0 %	16.96%	0.3342	16.8453
>= 100000	24.5 %	4.0 %	13.48%	0.2556	18.717
> 100000	0.0 %	100.0 %	53.91%		1

LR+ (Positive likelihood Ratio) (**LR-** Likelihood Ratio)

The receiver operating characteristic (ROC) curve of HRP2 RDTs and HRP2/ pLDH RDTs performance showed sensitivity against 1-specificity as the cut-off point's value moves from 0 to 1. ROC of 0.9008 was obtained for HRP2-RDTs and 0.8972 for HRP2/pLDH-RDTs showing high sensitivity for the two RDTs kits (Figure 4.2).

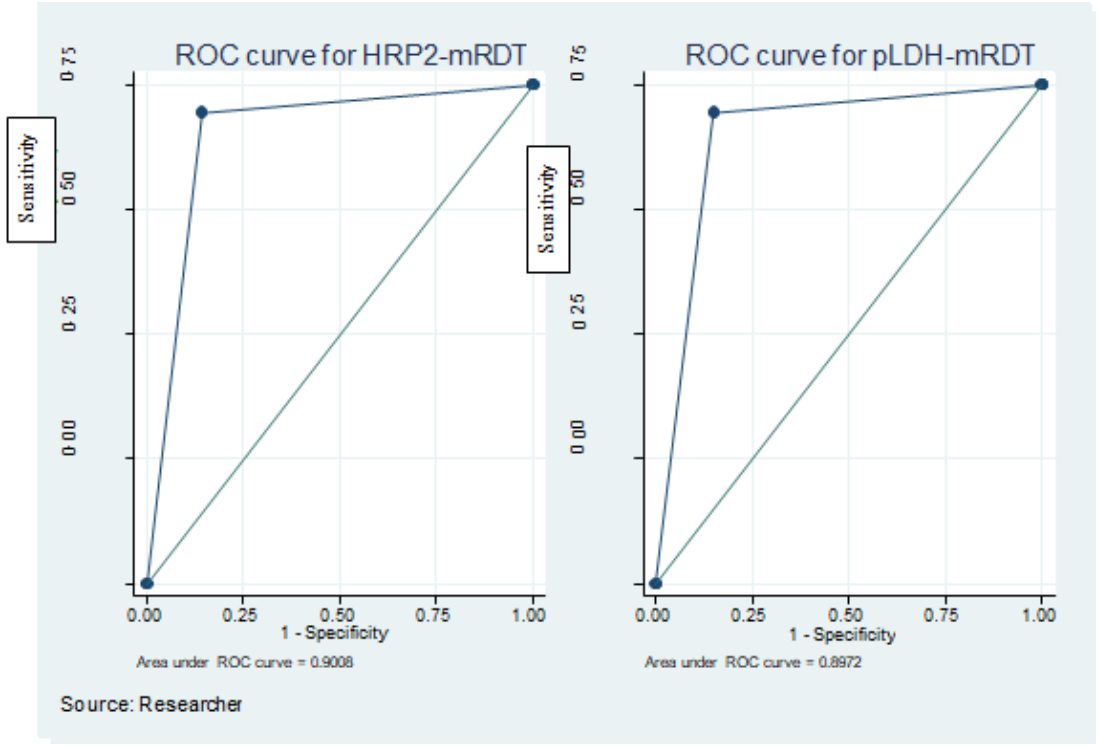


Figure 4.2 Receiver operating characteristic curve of the two malaria RDT performance with plotted points of parasite densities at selected cut-off points

4.3 Factors affecting the performance of RDTs used for diagnosis of malaria

4.3.1 Test device-related factors

In all the three sampled health facilities (Ratta, Miranga, and Manyuada), 18 (47.4%) of the health care providers reported that mRDTs were stored well. That is, the storage facilities were well ventilated with thermometer and monitoring chart updated. Out of 38 health care providers, 8 (21.1 %) did not achieve the recommended storage standards for mRDTs since the room temperature was not being monitored. There was no proper ventilation, and there was a lack of thermometer in these facilities. The results further show that only 12 (31.6 %) had an excellent storage area for mRDTs. Meaning they had all the requirements achieved for effective storage.

16 (42.1%) of the health care providers checked the expiry date of mRDTs before use, however, only 7 (18.4%) were able to monitor mRDTs expiry date regularly. Stock monitoring was reasonably achieved in all the sampled health facilities. 15 (39.5%) of the interviewed health care providers reported that malaria commodities' were availability (Figure 4.3).

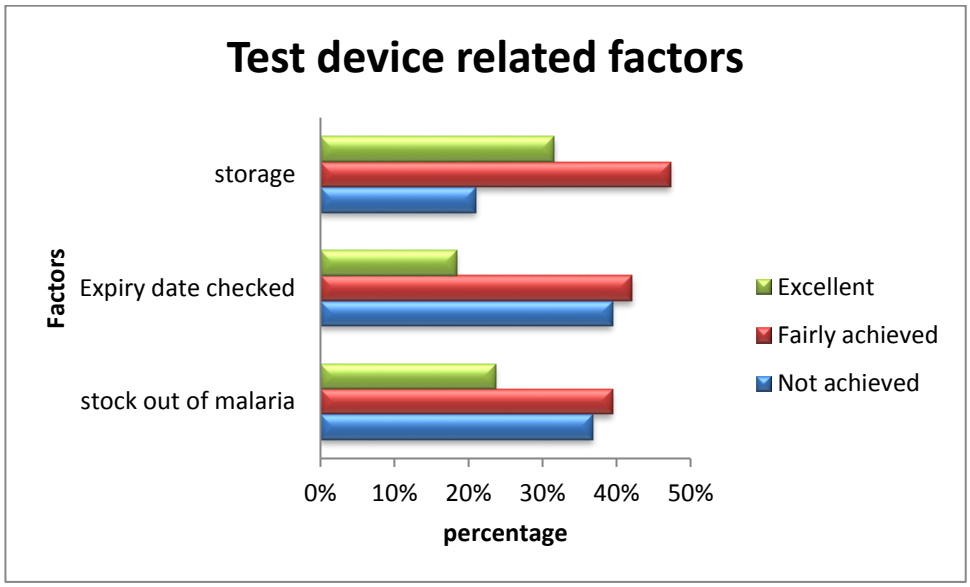


Figure 4.3 The achievement of test device-related factors

4.3.2 Factors associated with handling of the rapid diagnostic test kits

The results revealed that 12/22 (54.5 %) of community health volunteers (CHVs) training on malaria case management had been done in the last 2 years. For the facility health care workers, 2/6 (33.3 %) of the clinicians, 3/6 (50.0 %) of the medical laboratory officers and 2/4 (50.0 %) of the nurses were trained on malaria case management in the last two years.

Concerning checking of the expiry date of mRDTs, 12/22 (54.5%), 4/6 (66.7%), 4/6 (66.7%) and 3/6 (75.0%) of CHVs, clinicians, medical laboratory officers and nurses respectively, were able to routinely check the expiry date of mRDTs before use.

However, wearing new gloves when making a malaria diagnosis was excellently achieved by the health care workers.

Look at cleaning of the puncture site with sterilize alcohol swab before sample collection, 15/22 (68.1%), 6/6 (100.0%), 5/6 (83.3%) and 2/4 (50.0%) of CHVs clinicians, medical laboratory officers and nurses respectively cleaned the puncture site as expected before sample collection.

Considering adequate volume of blood collected during sample collection, the results obtained were as follows, 21/22 (95.5%) of the CHVs were able to collect the required amount of blood. 6/6 (100%) of the clinicians collected the required amount of blood during sample collection. Finally, 5/6 (83.3%) and 3/4 (75.0%) of medical laboratory officers and nurses respectively managed to collect the correct volume of blood during sample collection.

Finally looking at dispensing the required amount of buffer to cassette well when running the test showed that, 21/22 (95.5%), 6/6 (100.0 %), 5/6 (83.3%) and 4/4 (100.0%) of the CHVs, clinicians, medical laboratory officers and nurse respectively, were able to dispense the right amount of buffer as indicator by the manufacturer. On the other hand, 16/22 (72.7 %), 6/6 (100.0%), 5/6 (83.3%) and 2/4 (50.0%) of the CHVs, clinicians, medical laboratory officers and nurses respectively were able to dispense blood at the correct sample well (Figure 4.4).

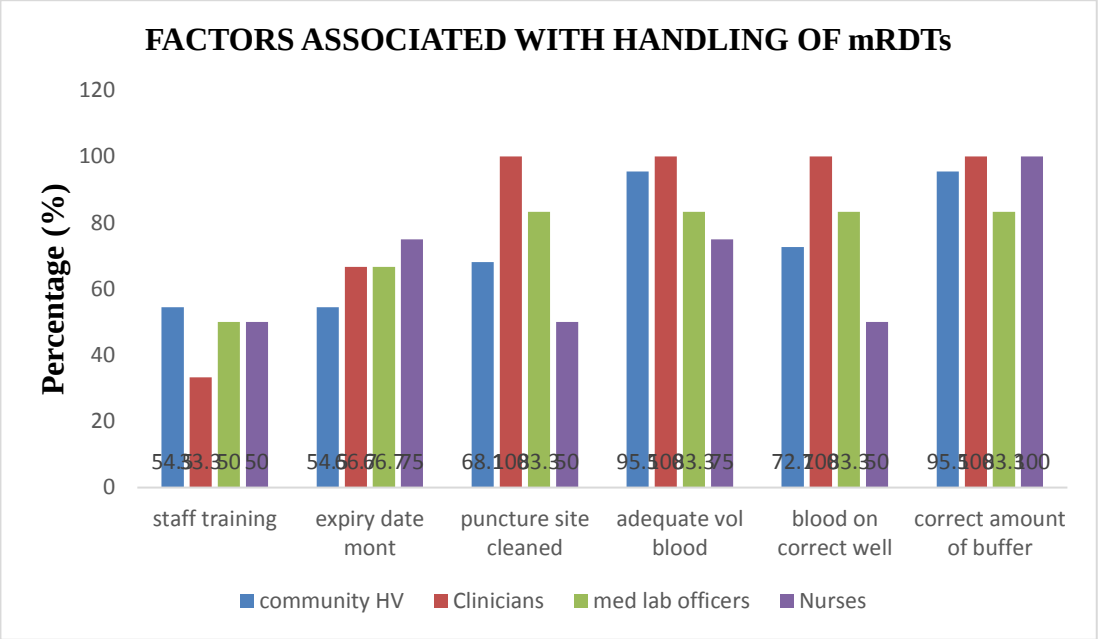


Figure 4.4 Factors associated with handling of mRDTs

4.3.3 Factors relating to quality control using mRDTs

Out of 38 health care workers interviewed, 27 (71.1%) were able to read and interpret test results correctly (figure 4.4). The study revealed that 18 (47.4%), were able to record test results correctly. However, the study revealed that no control wells were available to be used for internal and external quality control (IQC/EQC). It also revealed that no proficiency testing was being done to the health care workers (figure 4.4).

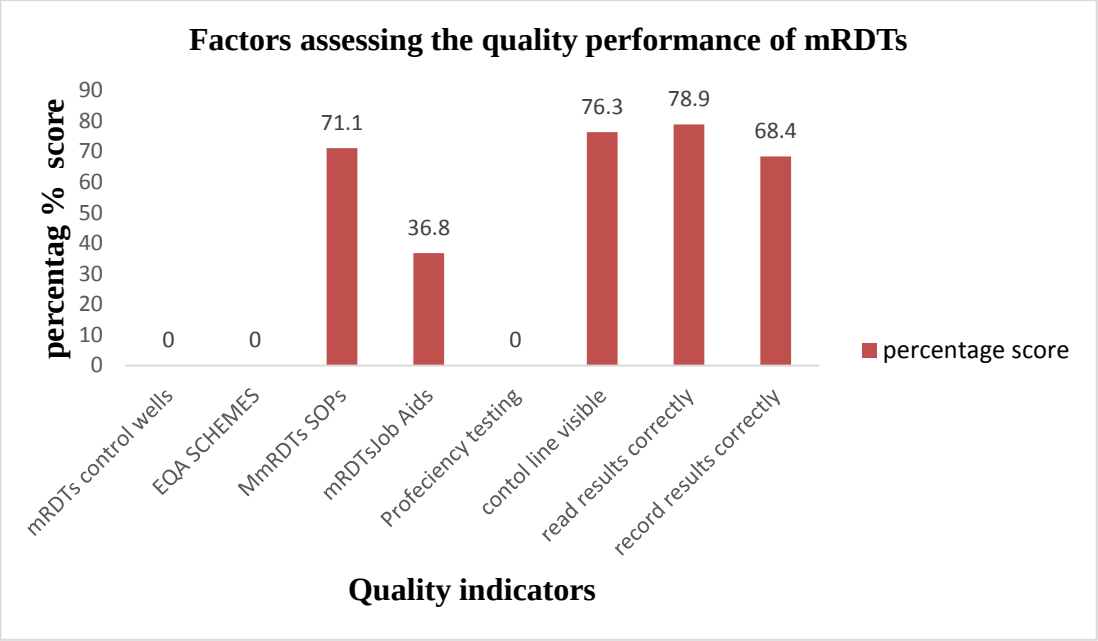


Figure 4.4 Factors assessing the quality performance of mRDT

Besides, the distribution of test kits evaluated per facility were as indicated below. A total of 223 whole blood sample panels were tested using the two mRDTs kits and microscopy as a reference method. Out of 223 diagnostic test kits evaluated, 92 (41.3%) were tested in the Ratta health facility, 65 (29.1 %) were tested in Miranga health facility, and 66 (29.6 %) were tested in Manyuada health facility (figure 4.5).

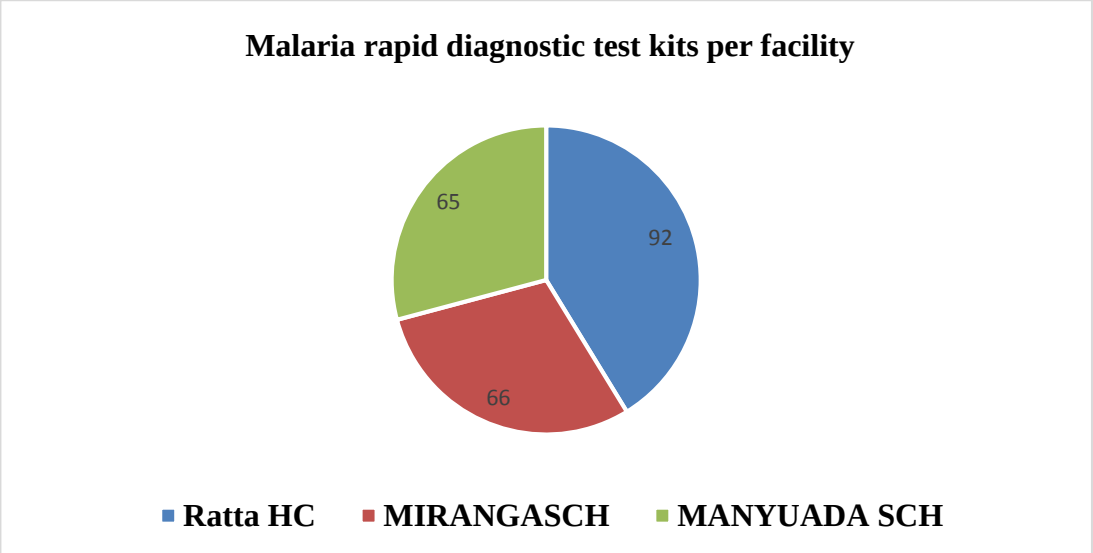


Figure 4. 5 Distribution of test kits evaluated per facility

CHAPTER FIVE

5.0 DISCUSSION

Quality diagnosis of *Plasmodium* species is important not only for establishing the correct treatment regimen but also for applying effective malaria prevention and control strategies in endemic regions where the three species of malaria parasites exist, as in Seme Sub County, Kisumu County. Failure to identify *Plasmodium* species could result in severe public health concerns due to inappropriate treatments, leading to recrudescence and even drug resistance (Kang *et al.,* 2017). Malaria prevention and control requires a high-quality diagnostic method to detect the parasite before prescribing antimalarial treatment following the WHO's indications. Malaria parasitological diagnosis targets treatment supports, the treatment response's characterization, and enables early identification of the parasite (Falade *et al.,* 2016).

5.1 Sensitivity, specificity and predictive values of HRP2mRDT and HRP2/ pLDH mRDTs

The sensitivity for the malaria rapid diagnostic tests obtained in this study was 94.4% and the specificity was at 85.7% for HRP2 based mRDTs and 85.0% HRP2/pLDH based mRDTs. This does not agree with the manufacturer's sensitivity of 98.0% and specificity of 97.5% for HRP2 and 99.0% sensitivity and 97.0% specificity of HRP2/pLDH. The possible reason is that like other biological tests, malaria RDTs are prone to deterioration through exposure to heat and humidity and manufacturing faults. However, the data from this study are consistent with a published work done in Kenya when RDTs are compared to microscopy which showed a sensitivity of >90% and specificity of >73% (Wanja *et al.,* 2016a). The agreement probably may be because the study took place in the same endemic area for malaria hence the similarity of the

study environmental conditions. Sensitivity and specificity differences among mRDTs can be due to several possible reasons, including exposure to extreme temperature and humidity that cause denaturation of antibodies, hrp2 gene polymorphisms and deletions, operational difficulties and human error during test performance. The results indicate that the mRDTs kits used in the study can detect correctly only with 217 out of 223 blood samples with malaria infection and will give a negative result in 196 out of the 223 blood samples without malaria infection. The false-positive may be due to the persistent antigen of the malaria parasite in the blood even after parasite clearance following adequate antimalarial treatment of the index cases as reported in the study done by Dalrymple and Al-Awadhi (Al-Awadhi *et al.,* 2021; Dalrymple *et al.,* 2018a).The false positives and negatives obtained in the current study can be attributed to low parasiteamia level, failure to adhere to SOPs, types of mRDTs used and probably due to test kits deterioration due to inappropriate storage condition.

The sensitivity and specificity reported in this study are yet to attain the ≥ 95% sensitivity and ≥90% specificity recommended World Health Organization (Berthod *et al.,* 2017). This low sensitivity is disadvantageous as it will impair control intervention since a fraction of the infected population will be left untreated especially if mRDTs is the only available diagnostic tool. The low sensitivity maybe because of the false negative obtained by mRDTs compared to the microscopy recorded in the study. The possible explanation for these findings may include a low parasite density below the threshold of mRDTs positivity (<100 asexual parasites/µl or <0.002% of red blood cells infected) (Mouatcho *et al.,* 2013). Other studies have also shown some degree of false-negative results for mRDTs because of Hyperparasiteamia, deletion or mutation of hrp2 gene, and the prozone effect, which is defined as false negative or false positive results in immunological reactions because of the excess of either

antigens or antibodies (Cheng *et al.*, 2014; Gendrot *et al.*, 2019; Kozycki *et al.*, 2017b). The effects of antigens and antibodies will eventually affect the test's sensitivity (Gillet *et al.,* 2009). The sensitivity obtained by this study was higher than for a study done in Nigeria. Myanmar endemic border in China also found that the HRP2 based rapid diagnostic test had a sensitivity of 89.7% and specificity of 98.3% compared to the gold standard microscopy method for the detection of malaria (Ogunfowokan *et al.,* 2020). Variation in sensitivity between the different studies may be attributed to differences in the types of mRDTs used or test methodology and skills of the microscopist.

The different mRDTs tested herein had relatively low specificity (85.7% and 85.0% for HRP2 and HRP2/pLDH respectively) in comparison to the study done in Ethiopia and Kenya, which had a specificity of 98.6% and 95.4% respectively (De Oliveira *et al.*, 2009; Feleke *et al.*, 2017). This was also far much lower compared to a study done in Kenyatta Hospital which had a specificity of 97% (Ndunda, 2019). The lower specificity in the current study could be attributed to the detection of HRP2 circulating antigens, which may persist in the blood several weeks after malarial treatment and low parasiteamia level. Ezeudu and colleagues also reported a sensitivity and specificity of 80.0% and 93.8% respectively (Ezeudu *et al.,* 2015). This was lower than the reports obtained in this current study. This can be attributed to different quality factors such as failure to follow standard operating procedure and testing guidelines that are capable of affecting the performance of mRDTs.

The persistent antigenaemia may have contributed to the low specificity recorded in the study. This also agrees with the work of Batwala in Uganda rural health centers, which reported that the overall specificity of mRDTs was lower than that of expert microscopy (Ogunfowokan *et al.*, 2020). The agreement is there probably because the

study areas are within the sub-Saharan Africa region where the storage and testing conditions are almost similar.

The percentage agreement of positive results of mRDTs and microscopy was at the perfect agreement of 89.1% for HRP2 based mRDTs and 88.7% for HRP2/pLDH based mRDTs. The expected agreement rate was 51.0% and 50.9%, respectively. This is consistent with a study done in Kenya which indicated that RDTs had a moderate measure of agreement (>80.1%) when compared to microscopy (Wanja *et al.*, 2016a). The agreement may be because this is a high endemic area; hence the parasiteamia density can be detected by both mRDTs and microscopy. The agreement on the sensitivity and specificity of the diagnostic test was statistically significant.

The implication of the low sensitivity in this study compared to the WHO set target is that in areas with low malaria parasiteamia, medical laboratory personnel should cross-check a negative result with the gold standard 'microscopy' and clinical understanding of the clinician to rule out possibilities of false negative with the mRDTs. The specificity of 85% in the study implied that mRDTs might be used in primary healthcare by community health volunteers to rule out the absence of malaria where microscopes are hardly seen or where the required expertise is lacking; however, for the health facilities with laboratory, the gold standard method should be used to ensure accurate positive results are obtained. The false-negative results can be caused by any or combination of the following. The procurement and use of poor quality mRDTs, poor transport and storage conditions for mRDTs with sustained exposure to high-temperature, operator errors during performance and interpretation of mRDTs results, and finally, deletion or mutation of *hrp2*.However, the possibility of the mRDTs detecting parasites with *hrp2* gene deletion needs to be explored. A review of HRP2 mRDTs is required given the reports of hrp2 gene deletion infection in Mali (Agaba *et*

al., 2019) and potentially in Ghana as suggested by Amoah *et al*. 2016 (Amoah *et al.,* 2016).

The positive predictive values on this study for HRP2 and HRP2/pLDH mRDTs were 81.0% and 80.2% respectively. The negative predictive values were higher than the PPV, for HRP2 it was 96.0% and for HRP2/pLDH mRDTs was 96.0%. The PPV obtained is slightly different from the findings of Falade *et al.,* 2016, who had PPV and NPV of 65.6% and 86.1%, respectively. The positive predictive value of 81.0% means that the kit can detect malaria with a precision of 81.0%. In contrast, the negative predictive value of 96.0% means that the mRDTs are good in ruling out malaria, thus giving the clinician the confidence that the negative test excluded malaria in about 96.0% of cases. The differences in predictive values may be attributed to poor transport and storage conditions for mRDTs, with sustained exposure to high temperature and operator errors during the performance and/or interpretation of mRDT results.

5.2 Quantitative limit of parasite density cut off points

The study reveals that the performance of mRDTs was at the different cut off points. It shows that the minimum parasite density detected by HRP2 and HRP2/pLDH mRDTs is 80 parasites/µl of blood. This is higher than the detection limit given by the test kits manufacturer who gave the lower detection limit to be 50 parasites/µl of blood. The differences in detection limit depends on the accuracy and sensitivity of the microscopist expert used. The two mRDTs evaluated in this study had acceptable performance in samples with parasite densities from 80 to >100,000 parasites/µl of blood. The detection limit obtained is slightly higher than the study done in Ghana, which revealed a parasite density detection limit from 25 parasites/µl of blood to 96

parasites/µl of blood (Adu-Gyasi *et al.*, 2018). Another study recorded sensitivity of HRP2/pLDH rapid diagnostic tests remained relatively low for *P.falciparum*, detecting a lower limit of 200 parasites/µl of blood compared with HRP2 based rapid tests which detected 50 parasites/µl of blood (Slater *et al.*, 2019). The differences in parasite density cut off points can be attributed to test procedure, factors affecting biological tests and the technical competency.

The sensitivity of mRDTs in this study was shown to increase with an increase in parasite density. This is true because the higher the parasiteamia level the greater the percentage agreement of positive results of mRDTs with microscopy. It, therefore, means that the parasite density is related to mRDTs positivity. This implies the inability of RDTs to reliably detect malaria parasites at low parasite densities. Therefore, it means that the parasite density is directly related to the mRDTs positivity rate. The result agrees with a study done in Nigeria (Ogunfowokan *et al.*, 2020). It is assuring to note that the kits with targets to pLDH detecting antigens in addition to HRP2 performed well compared with only HRP2 antigen detecting kit at low parasite densities. The RDT kits, particularly with pLDH detecting antigens, can be considered for use to screen and detect infections with *Plasmodium falciparum*. These findings highlight the importance of treating patients with low-density malaria parasiteamia and support interventions addressed to eliminate submicroscopic infections (Berzosa *et al.*, 2018).

The discriminating power of RDTs for the detection of malaria was further investigated by the area under the receiver operative characteristics (ROC). It was found that RDT (area under the diagnostic curve –AUC: HRP2 0.9008 and HRP2/pLDH 0.8972) was more effective in predicting malaria infection, This is in

agreement with the work of (Djimde *et al.,* 2016) in Mali, mRDT which recorded a higher area under the curve ROC curve 0.97.

The implication of this study findings is that these mRDTs are not able to detect parasites below 80 parasites/µl of blood. This might lead to delay in treatment which might result to death especially in children under 5 years and pregnant women. The good agreement between the tests obtained can be attributed to the high malaria endemic zone where the study took place. Although mRDTs are used as diagnostic methods, personnel working in the laboratory should never abandon microscopy because it is the gold standard in endemic areas. Besides, microscopy allows the calculation of parasitic densities, identifying all species and is cheaper than other methods.

5.3 Factors affecting the performance of malaria rapid diagnostic tests

It is widely established that the key factor in improving the diagnosis of malaria is the availability of mRDTs in health facilities. This study found that rapid diagnostic kits were available in 39.4% of the health facilities this was higher than a study done in Enugu state where 31% of health facilities had mRDTs and lower than a study done in Ogun state that reported mRDTs was available in 50.7% of the health facilities(Boyce *et al.,* 2017). This, however, is less than the WHO average availability which is said to be inadequate if it falls below76%. Stock out of testing commodities can be due to erratic supplies from KEMSA, improper documentation and reporting of quantity used and probably due to inadequate information needed during the forecasting and quantification period. Planning to have adequate stock, especially over the peak transmission period, is vital to preventing stock-outs. Liaison with the Sub County medical laboratory officer is crucial to ensure a steady stream of stock replacement.

The manufacturer's recommendations for storage of mRDTs are 2^{o}c to 30^{o}c (Diagnostic package insert). The study revealed that effective storage was at 50.0%, which means that temperature monitoring and storage conditions were not effectively monitored. Poor storage is basically due to the different level of health care systems. The primary level is not able to offer the expected storage conditions for the mRDTs and this can only be achieved in level 2 health care. Like other biological tests that rely on antibody-antigen interactions, current malaria RDTs deteriorate more quickly on exposure to moisture (humidity) and high temperature. Poor storage might interfere with the integrity of the test kits leading to the poor performance of the mRDTs. Increases in temperatures above 30^{o}c can affect the overall performance of mRDTs. Therefore it, becomes important that room temperatures are maintained below 30^{o}c, and temperature monitoring is taking place regularly in the mRDTs storage room of all the health facilities and community testing. Those rooms that exceed temperatures of 30^{o}c should be cooled with air conditioning or similar cooling equipment, this being dependent on financial resources available to local health authorities in the study Sub County, this agrees with the study done by Moody (Obeagu *et al.*, 2018).

There was huge uncertainty in the quality control and accuracy of mRDTs by end-users. The study revealed that health care workers did not use positive control for wells for external quality control, and the test kit verification was not being done and there was no proficiency testing in place for health care workers. The health facilities depends on the inbuilt internal quality control. It is important to consider the use of positive control wells and temperature monitoring to assure the quality of the rdts and to build the confidence of the users on mRDT (Bell *et al.*, 2017). Discrepancies between microscopy results and end-user findings on mRDTs are a concern. The discrepancies point to two challenges, either the test kits were not working, or the end-

user was not using the kits correctly, as reported by Rennie and Moonasar (Boyce, 2017). Adherence to the standard operating procedures was below the expected performance target which 100 % and this contributes to the low performance of mRDTs.

Another factor influencing mRDTs utilization and performance found in this study was training. This is similar to previous studies that showed that training of health care workers on mRDTs improves health care workers performance with increased likely hood of adherence to malaria treatment guidelines (Opoku, 2018). External training was not being conducted for the end-users regularly as expected. On job training also was not based on the standard operating procedures using standardized materials and methods. There is also uncertainty on the quality of existing on the job training for mRDTs because even the supervisors had not been refreshed. Various studies have documented significant variation between end-users in both preparation and interpretation (Boyce *et al.,* 2017; Harvey *et al.,* 2017; Mathison *et al.,* 2017). A standardized training guide for on job training may need to be considered (Kosack *et al.,* 2017). Although package inserts are useful, it would be easier for end-users to have bench job aids so that the test procedures can be easily visible and interpretation of results easily read, especially during busy periods and late in the night. Preparation and interpretation can also be affected by manual dexterity, visual acuity and available lighting. To perform the tests in a realistic test environment the training and previous experience of the end-users is necessary. The uncertainly about the skills of use of mRDTs from both the health facilities users and the community health volunteers is a cause for concern. Proficiency testing should be considered to evaluate the skills of the end-users of mRDTs scientifically.

The key challenges for malaria mRDTs in the health facilities are frequent stock-outs, the accuracy of the kit, the proficiency of the end-user, and quality control to ensure that the kits are working well to monitor the diagnostic performance. This conquers with a study done in Zamfara State on predators of malaria rapid diagnostic tests utilization among the health care workers (Usman *et al.*, 2018). The implication of this finding that it may lead to misdiagnosis which might delay treatment and in worse cases it might lead to death.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

(i) The study showed that both HRP2 and HRP2/pLDH mRDTs kits had equal sensitivity, while the difference in specificity between the two was deemed insignificant. The HRP2 mRDT had a higher positive and negative predictive value compared to HRP2/pLDH mRDTs, even though the mRDTs gave an acceptable agreement with the reference test method.

(ii) The quantitative limit of parasite density cut off points for both HRP2 and HRP2/pLDH mRDTs was at 80 parasites/µl of blood.

(iii) Factors affecting the performance of mRDTs in Seme Sub County included unsatisfactory storage, inadequate training for health care workers, and failure to adhere to standard operating procedures and lack of quality assurance schemes.

6.2 Recommendation

(i) The study recommends the improvement of the HRP2 based mRDTs to be done by incorporating other antigens such as Aldolase and plasmodium lactate dehydrogenase to improve their sensitivity and specificity.

(ii) The study recommends that mRDTs should not be used in low parasite density endemic zones this is because they are unable to detect low parasiteamia level.

(iii) Temperature monitoring of the storage areas for MRDTs, regular training of staff, quality assurance measures and proficiency testing is

recommended to ensure that quality results is obtained when mRDTs is used for malaria diagnosis.

6.2.1 Recommendations for action

(i) We recommend scaling up malaria microscopy diagnostic sites and data on diagnostic performance of mRDTs test kits is important information to guide in diagnostic tool selection by the national malaria control programs.

(ii) Evaluation of factors affecting the performance of rapid diagnostic test needs to be done frequently to ensure that the necessary conditions required for the effective performance of the RDTs are in place.

(iii) The study recommends provision of proficiency samples panels to be provided to the mRDTs end users quarterly to assess the competency of the health care workers.

6.2.2 Recommendation for further research

(i) After the supplying of mRDT from Kenya medical supplies urgency in health facilities, subsequent studies should be done on their impact and cost-effectiveness in providing quality diagnosis at the point of care where these mRDTs are used.

(ii) There is need of further studies on the factors influencing the effective use of mRDTs and the ways to improve the use of mRDTs are needed in different areas where RDTs are used.

(iii) The study recommends determination of genetic variation to detect the existence of hrp2 gene deletion type of *plasmodium* parasites which cannot be detected by HRP2 based mRDTs.

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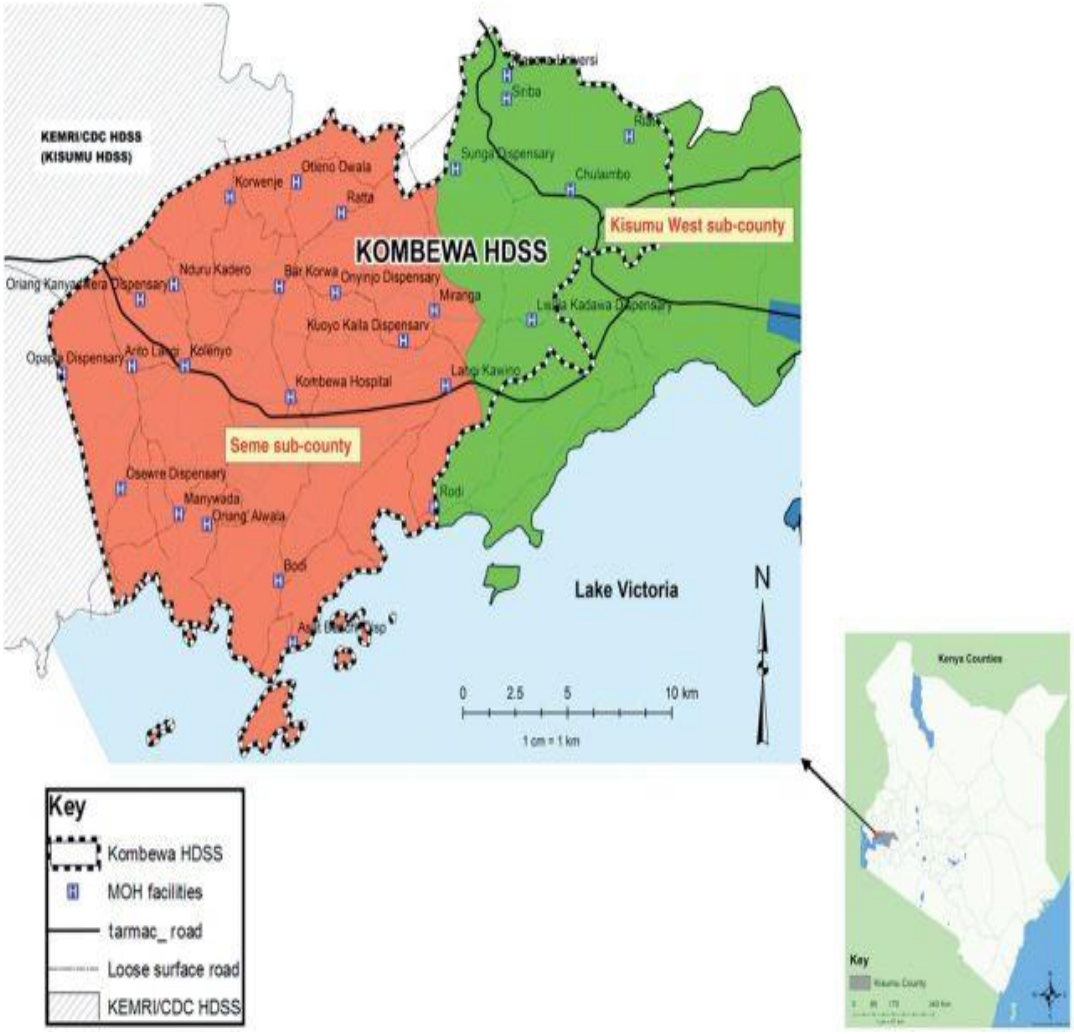
APPENDICES

APPENDIX I: A MAP SHOWING THE STUDY AREA



APPENDIX II: Observation and questionnaire Checklist on RDT Assessment

For each step below, circle one if the Health Care worker performed the step correctly.

Circle two if the health care worker performed the steps incorrectly.

Circle three if the health care worker skipped the step.

Name of the supervisor----------Supervisor phone number----------

Type of health care provider being observed----------Date of assessment----------

No	ITEM	SCORES		
1	Is the staff trained in malaria Rdts in the last two years?	1	2	3
2	Did the facility experience a stock out of malaria rdts for seven or more consecutive days during the last three months?	1	2	3
3	Are the mRDTs sops and bench aids available?	1	2	3
4	Are the mRDTs sops and bench being used during testing?	1	2	3
5	Does the facility conduct RDT verification exercise?	1	2	3
6	Does the facility use malaria control wells?	1	2	3
7	Is expiry date of the mRDTs checked before use?	1	2	3
8	Is the cassette labeled with patient's name/id number?	1	2	3
9	Is cassette labeled with date, start and stop time?	1	2	3
10	Patient identified and identification information recorded in register?	1	2	3
11	glove worn	1	2	3
12	New gloves worn	1	2	3
13	puncture site cleaned with sterile alcohol swab and allowed to air dry	1	2	3
14	An adequate volume of blood is collected	1	2	3
15	Blood dispensed in correct well of RDT device	1	2	3
16	buffer applied to correct well of RDT device	1	2	3
17	appropriate amount of buffer dispensed into well	1	2	3
18	were the test results positive or negative	Pos	Neg	IN
19	If negative did the worker wait the recommended amount of time (according to manufacturer's instruction) to rule out a positive test result?	1	2	3
20	control line visible	1	2	3
21	Reads test results correctly (supervisor verifies results)	1	2	3
22	Records results correctly in the register	1	2	3
23	Is the storage area well ventilated?	1	2	3
24	Is the storage area having thermometer and temperature monitoring chart update?	1	2	3

APPENDIX III: Consent Form

TITLE: FIELD EVALUATION OF DIAGNOSTIC PERFORMANCE AND FACTORS AFFECTING MALARIA RAPID DIAGNOSTIC TEST IN SEME, KISUMU COUNTY

You are welcomed to take in a research study to be conducted by a master's student from the university of Masinde Muliro Kakamega campus. You must be a patient attending the hospital and suspected to have malaria. Participation is voluntary and you are expected to take enough time to read the information sheet. You area allowed to discuss with your family members and friends. A copy will be provided for you to carry home.

AIM OF THE STUDY

The study is evaluating the diagnostic performance and assessing the factors affecting rapid diagnostic test in Seme Sub County.

If you complete and return this form it will constitute consent to participate in this research project.

PROCEDURES: *The procedure requires you to donate 2mls of venous blood sample. This will take 5 minutes and it will take place in the hospital as the normal routine hospital procedures. Injury and harm will be minimize by using a trained phlebotomist.*

POTENTIAL BENEFITS TO RESEARCH PARTICIPANTS/OR COMMUNITY

The research will not attract any benefit from your participation nor will the research provide a benefit to you.

REWARD FOR PARTICIPATION

The study will not attract any financial reward to the research participants

POTENTIAL CONFLICTS OF INTEREST

The investigators of this research do not have any financial interest in the in the product or commodities being studied.

CONFIDENTIALITY*: Any information that is obtained in connection with this study that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. The information collected about you will be coded using initial numbers (unique identifiers). The data will be stored in the investigators password protected computer. When results of the research are published or discusses in scientific conferences, Information about the participants will not be revealed.*

***PARTICIPATION AND WITHDRAWAL*:** Participation is voluntary and *You can choose whether to be part in this study or not, if you volunteer to be in this study, you may withdraw at any time without consequences of any kind or investigator may withdraw you from this research if circumstances arise which warrant doing so.*

***ALTERNATIVES TO PARTICIPATION**: Your alternative to participation is not to participate.*

***RIGHTS OF RESEARCH SUBJECTS** You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. If you have any questions about your rights in the study. Consult cellineokuta@gmail.com (0722477839)*

***IDENTIFICATION OF INVESTIGATORS: If** you have any questions or concern about the research, please feel free to contact the investigator and the faculty advisor*

Masters candidate *Faculty advisor*

CELLINE ATIENO OKUTA
P.O.BOX 60 KOMBEWA
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0722477839

Dr. CHRISTINE WANJALA
MASINDE MULIRO UNIVERSITYOF SCIENCE AND TECHNOLOGY
MEDICAL LABORATORY SCIENCES DEPT
cwanjala@mmust.ac.ke
0724593853

If you have any questions concerning your rights as a research participant call The Secretary/Chairperson MMUST – IERC Tel:056-31375 , Email ierc@mmust.ac.ke

APPENDIX IV: Minor Assent Document
Research Title: ***FIELD EVALUATION OF DIAGNOSTIC PERFORMANCE AND FACTORS AFFECTING MALARIA RAPID DIAGNOSTIC TEST IN SEME, KISUMU COUNTY***

Investigator: CELLINE ATIENO OKUTA

We are doing a research study about field evaluation of diagnostic performance and factors affecting rapid diagnostic tests kits commonly used in the Sub County. The importance of a research is to gives us more information about the target population.

Procedure, if you opt to be part of this study, you will be expected to donate 2mls of venous blood sample. The procedure will take 5 minutes in the health facility as part of the normal routine health facility procedures.

Danger and pain, there will be no harm and pain involved, however a trained phlebotomist will be used to minimize the risks. Little pain may be felt during sample collection.

This study will not offer any direct benefit, however the results will benefit the entire community because it will inform the policy makes on the most appropriate diagnostic tool to use. A benefit means that something good happens.
Voluntary participation. If you opt out of this research study, you are allowed to withdraw and only benefit from the normal routine medical services that brought you to the hospital. This study is voluntary and you are not forced to be in it if you don't want to. You are allowed to drop at any stage. Your parents have been notified about the study too.

The report of the study and lessons learnt will be documented. This report will not reveal your identity or your role in the study.

Please sign your name if you accept to participate in the study.

I, _____, want to be in this research study.

Signature _____ Date _____

For any question or concerns or complain if not treated well as a research participant please call, The Secretary/Chairperson MMUST – IERC Tel:056-31375 Email ierc@mmust.ac.ke

APPENDIX V: Statement of Intent to Maintain Confidentiality
Title: ***FIELD EVALUATION OF DIAGNOSTIC PERFORMANCE AND FACTORS AFFECTING MALARIA RAPID DIAGNOSTIC TEST IN SEME, KISUMU COUNTY***

Research staff

As a research assistant and a member of this research team, I acknowledge that I may have access to confidential information about study site and participants. By signing this statement, am indicating my understanding of my responsibilities to maintain confidentiality and agree to the following;

1. I acknowledge that names and any other unique identifier information about study sites and participants are completely confidential.
2. I agree not to divulge or otherwise make known to unauthorized person or to the public any information obtained in the course of the research project that could identify the persons who participate in the study.
3. I understand that all information about study sites or participants obtained or accessed by me in the course of my work is private and confidential.

Name of the research staff _____

Signature_____DATE_____

Witnessed by_____

Signature_____DATE_____

APPENDIX VI: Results Tracking Log
Facility

STUDY NO	MICROSCOPY RESULTS	HRP2 BASED mRDTs	HRP2 COMBINED WITH PLDH BASED mRDTs	COMMENT

APPENDIX VII: Directorate Postgraduate Research Approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

17th August, 2020

Celline Atieno Okuta
HML/G/01-57290/2016
P.O. Box 190-50100,
KAKAMEGA.

Dear Ms. Okuta ,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Master's proposal entitled: ***"Comparative Study Between Rapid Diagnostic Tests and Microscopy for Diagnosis of Malaria in Seme, Kisumu County"*** and appointed the following as supervisors:

1. Dr. Christine Wanjala - SPHBS&T MMUST
2. Mr. Fidelis Mambo - SPHBS&T MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Ph.D thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

DEAN
SCHOOL OF GRADUATE STUDI
MASINDE MULIRO UNIVERSIT
OF SCIENCE & TECHNOLOGY

Dr. Consolata Ngala
DEPUTY DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

APPENDIX VIII: Institutional Ethics Review Committee Approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190-50100
Kakamega, Kenya

Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 Vol 3 (02) **Date:** 14th October, 2020

Celline Atieno Okuta
Masinde Muliro University of Science and Technology,
P.O. Box 190-50100.
KAKAMEGA.

Dear Ms Okuta,

RE: Comparative study between rapid diagnostic tests and microscopy for diagnosis of malaria in Seme, Kisumu County, Kenya- MMUST/IERC/127/2020

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with no further revisions) the above Referenced application for one year.
This approval is valid from **14th October 2020** through to **14th October 2021**. Please note that authorization to conduct this study will automatically expire on **14th September 2021.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **14th September 2021.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC Secretariat by **14th September 2021.** You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/event resulting from the conduct of this study must be reported to MMUST IERC. Also note that you are required to seek for research permit from NACOSTI prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

APPENDIX IX: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 296340

Date of Issue: 25/November/2020

RESEARCH LICENSE

This is to Certify that Miss.. Celline Okuta Atieno of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Kisumu on the topic: COMPARATIVE STUDY BETWEEN RAPID DIAGNOSTIC TESTS AND MICROSCOPY FOR DIAGNOSIS OF MALARIA IN SEME, KISUMU COUNTY, KENYA for the period ending : 25/November/2021.

License No: NACOSTI/P/20/7321

296340

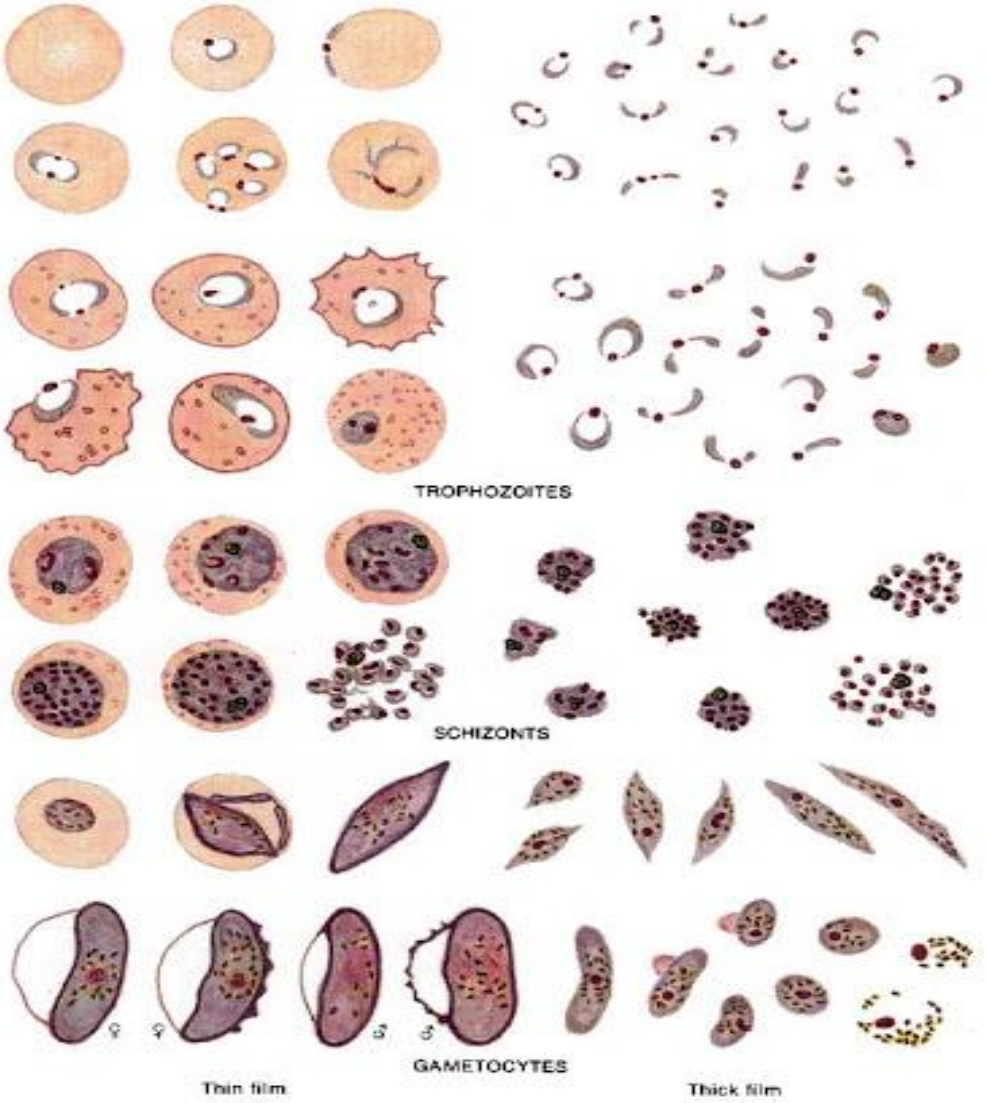
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX X: DIAGNOSTIC STAGES OF MALARIA PARASITE



APPENDIX XI : PUBLICATION OF OBJECTIVE TWO

Medicine and Medical Science MMS
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Research Article

Comparative Study between Rapid Diagnostic Tests and Microscopy for Diagnosis of Malaria in Seme, Kisumu County, Kenya

***Celline Okuta[1], Hellen Ogolla[2], Fidelis Mambo[1], Christine Wanjala[1]**

[1]Department of Medical Laboratory, Masinde Muliro University of Science and Technology, Kakamega, and Ministry of Health Seme Sub County, Kenya,

[2]Ministry of Health Kisumu County, Department of medical laboratory Services, Kisumu, Kenya

***Corresponding Author Email:** cellineokuta@gmail.com

Malaria infection is a global problem accounting for a 25% mortality rate annually, management and control of malaria involves accurate diagnosis and treatment. The study compared the performance of rapid diagnostic tests and microscopy as used for the diagnosis of malaria in Seme Sub County, Kisumu County. The cross sectional study was conducted in three purposively selected health facilities. A total of 230 participants were randomly selected to participate in the study. Blood samples were collected by a trained phlebotomist from the participants who had given consent to participate. The samples were screened for malaria using both microscopy as a gold standard and two Rapid diagnostic tests (Histidine Rich Protein (HRP2), and Combined HRP2 and parasite lactate dehydrogenase (PLDH) to determine the performance of RDTs. The results revealed that, the sensitivity, specificity, positive predictive values and negative predictive values using microscopy was found to be 94.44%, 85.71%, 80.95%, 96.00% for HRP2 and 94.44%, 85.00%,80.19% ,95.9% for pLDH RDT respectively. There was a significant level of agreement between microscopy and HRP2 RDTs of 89.13% (p-value <0.001) and between microscopy and pLDH RDTs of 88.70% (p-value <0.001). The low sensitivity below the WHO recommendation of ≥95% indicates the need to improve the sensitivity of the mRDTs kits in malaria management, where trained microscopists for malaria diagnosis are not available .The findings are important in informing the ministry of Health and the malaria control unit to improve on the malaria diagnosis techniques. Assist policymakers in post market surveillance of the mRDTs currently in use.

Keywords: *malaria diagnosis, rapid diagnostic test, sensitivity, specificity, predictive values.*

INTRODUCTION

Malaria is a parasitic infection caused by protozoan from the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. It is the highest contributor to morbidity and mortality in the developing part of the world (Nonvignon *et al.*, 2016).In order to reduce morbidity and mortality rate resulting from malaria infection, it's important to ascertain that the diagnostic techniques being used are accurate. Malaria is typically diagnosed using microscopy, and this is accepted and regarded as the reference method "gold standard"(Endeshaw *et al.*, 2008). Microscopy is important because within a few hours of collecting the blood, the test can provide valuable information. First and foremost it can determine that malaria parasites are present in the patient's blood. Once the diagnosis is established, usually by detecting parasites in the thick smear, the medical laboratory officer can examine the thin smear to determine the malaria species and the parasitemia, or the percentage of the patient's red blood cells that are infected with malaria parasites. The thin and thick smears are able to provide all the three vital pieces of information to the doctor to guide the initial treatment decisions that need to be made acutely. Different types of RDTs kits have been developed for the diagnosis of malaria infection in both malaria non-endemic and endemic zone due to challenges of availability of skilled, laboratory personnel and logistics to microscopy in several endemic countries, as part of malaria management and