HISTIDINE RICH PROTEIN II AND LACTATE DEHYDROGENASE LEVELS IN SALIVA AND BLOOD IN ACUTE MALARIA CASES IN KISUMU, WESTERN KENYA

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CELL AND MOLECULAR BIOLOGY

SCHOOL OF PHYSICAL AND BIOLOGICAL SCIENCES

MASENO UNIVERSITY

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DECLARATION

I declare that this is my original work and has never been presented for award of degree in any University.

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We (supervisors) confirm that the work reported in this thesis was carried out by the above candidate under our supervision.

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ACKNOWLEDGEMENT

First, I thank God for His grace and favour. I also thank my academic supervisors Dr. Cyrus Ayieko and Dr. Patrick Onyango for their guiding me through the entire study. This research project would not have been possible without their mentorship, encouragement and immense support. I also appreciate Prof. David Miruka Onyango for enabling me to carry out molecular experiments at Maseno University postgraduate laboratory at the Department of Zoology. I sincerely appreciate all the laboratory techinicians at the department of Zoology for their technical assistance and moral support during the entire period of carrying out my research. Special appreciations goes to Mr. Philip N Ochieng, Walter Odipo, Barnabas Muya, Joab Pira and Julius Adundo for guiding me through my experiments.

Moreover, I am grateful to Kenya Medical Research Institute (KEMRI-KISIAN laboratory director, Dr. Erick Mwok. Special thanks goes to my supervisor Dr. Sydney Ogolla for guiding me through the sample collection process. In addition, I sincerely thank Mr. Ben Sati, a post graduate student at Maseno University who was assisting me with sample collection at Chulaimbo sub-county Hospital. I extend sincere gratitude to the KEMRI-CWRU Case Western Reserve University Field staff, Oluoch Caleb, Otieno Harisson, David Midem and Ochieng Seline and laboratory staff, Dr. Sydney Ogolla, Emily Koech, Kevi Waomba, Stella Chumbe and Ben Okeyo. I am also grateful to all the volunteered study participants in this study.

I am also grateful for the members of staff at International Livestock Research Institute (ILRI) for granting me the opportunity to analyze my samples in their laboratory. Special thanks goes to my supervisor Ms Getrude Wekesa for guiding me through the analysis process and Regina Njeri who was in charge of the logistics and my welfare at the institute.

Finally, I thank my family members and friends for their support and prayers throughout the study period.

DEDICATION

To God almighty, my brother John Omingo and his wife Lucy Omingo, my sister Prisca Omingo and friend Sylvia Onchaga for their immense support throughout the study period.

ABSTRACT

Globally, malaria is the leading cause of death and economic burden. Diagnosis currently relies on microscopy and blood-based rapid diagnostic tests (RDTs). However, both methods are invasive, which increases the risk of accidental infection and is painful. Non-invasive approaches are thus required. *Plasmodium falciparum* Histidine Rich Protein-2 (*Pf.*HRP-2) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf.*LDH) are parasite enzymes released into the host blood during clinical malaria infection and have been demonstrated in saliva though with inconsistent results. This may be because most of these studies have been performed in low malaria endemic zone. This study seeks to determine the levels of these antigens in blood and saliva in a high malaria endemic zone and determine their relationship with parasite density. On the other hand, the performance of blood-based RDTs is dependent on parasite density, which in turn decreases with age. The present study determined the performance and efficiency of detection of *P. falciparum* antigens levels in saliva and blood as potential biomarkers in the diagnosis of malaria infection using blood-based RDT and saliva-based ELISA assay across ages. Specifically, the study determined the relationship between levels of *P. falciparum* antigens in saliva and blood, and parasite densities, clinical malaria and age in individuals of varying ages with acute malaria: The correlation between the antigen levels in blood and saliva and the sensitivity, specificity, positive and negative predictive values for detection of *P. falciparum* antigens. This was a cross-sectional study involving participants presenting with clinical malaria at Chulaimbo Sub- County hospital. Levels of *P. falciparum* antigens in saliva and blood were measured using ELISA. Generalized linear model was done to assess the relationship between the levels of *P. falciparum* antigens to parasite densities, clinical malaria and age. Correlation between levels of *Pf.* HRP-2 and *Pf.*LDH in blood and saliva were evaluated using Pearson's correlation. Sensitivity/specificity of the blood-based rapid diagnostic tests as well as blood and saliva-based Elisa assay were calculated. Parasite density did not predict *Pf.*HRP-2 in plasma and saliva (p=0.974) and (p=0.635) respectively. Also, parasite density did not predict *Pf.*LDH in plasma and saliva (p=0.570) and (p=0.315) respectively. In addition, clinical malaria did not predict *Pf.*HRP-2 in plasma and saliva (p=0.179) and (p=0.895) respectively. Equally, clinical malaria did not predict *Pf.*LDH in plasma and saliva (p=0.291) and (p=0.272) respectively. In contrast, age significantly predicted *Pf.*HRP-2 levels in plasma (p=0.00) but not in saliva (p=0.580). On the other hand, age did not significantly predict *Pf.*LDH levels in both plasma and saliva (p=0.406) and (p=0.764) respectively. Moreover, there was no significant relationship in the levels of *Pf.*HRP-2 and *Pf.*LDH in plasma and saliva (r=-0.235, p=0.104) and (r=-0.0235, p=0.104) respectively. Sensitivity of detection using blood-based RDTs in children and adults was found to be (98%) and (100%) respectively. Specificity was (28%) in children and (83%) in adults. Sensitivity of *Pf.*HRP-2 detection in children by ELISA was 78% in plasma and 13% in saliva. *Pf.*LDH in children was detected at a sensitivity of 15% in plasma and 7% in the saliva. In adults, *Pf.*HRP-2 had a sensitivity of 75% in plasma and 50% in saliva. Specificity of *Pf.*HRP-2 was 40% in plasma and 95% in saliva of children. *Pf.*LDH was detected at 100% specificity in both plasma and saliva of children and adults. Measurement of *Pf.*HRP-2 and *Pf.*LDH in plasma and saliva may not be a good proxy measure of infection and clinical disease in populations exposed to endemic malaria, However, *Pf.*HRP-2 can be used to measure cumulative exposure. Measurement of *Pf.*HRP-2 and *Pf.*LDH in saliva cannot substitute for the measurement in plasma hence plasma-based assays are more reliable. The lower sensitivity of *Pf.*HRP-2 in both plasma and saliva in holoendemic areas may not be improved using saliva samples prompting the need for further research.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATP	Adenosine Triphosphate
CI	Confidence Interval
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunosorbent Assay
GLM	Generalized Linear Model
GTS	Global Technical Strategy
HRP-2	Histidine Rich Protein-2
HIV	Human Immunodeficiency Virus
HDSS	Health And Demographic Surveillance System
IQR	Interquartile Range
KEMRI	Kenya Medical Research Institute
KH_2PO_4	Potassium dihydrogen phosphate
LDH	Lactose Dehydrogenase
LOD	Limit of detection
MAF	Malaria Attributable Fractions
MUERC	Maseno University Ethical Review Committee
Na_3OV_4	Sodium orthovanadate
Na_2HPO_4	Disodium hydrogen phosphate
NaOH	Sodium Hydroxide

PCR	Polymerase Chain Reaction
PMSF	Phenylmethylsulphonyl fluoride
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline-Tween
qPCR	quantitative Polymerase Chain Reaction
RBCs	Red Blood Cells
RDTs	Rapid Diagnostic Tests
SD	Standard Deviation
WHO	World Health Organization
WBC	White blood cells

LIST OF DEFINITION OF TERMS

Term	Definition
Sensitivity	The ability of a test to correctly identify patients with a disease
Specificity	The ability of a test to correctly identify people without the disease
Positive predictive values	The proportion of true positive
Negative predictive values	The proportion of true negative
Parasite density	Number of asexual forms of parasites relative to a blood volume
Prozone effect	A false negative test result due excess of either antigens or antibodies
Performance	Responsiveness or stability of a test
Biomarkers	Cellular, biochemical, or molecular alterations measurable in biological samples indicating any biological pathogen
Signal	Ability of an instrument to receive, process and transmit signals
Noise ratio	Ratio of the light signal to the nose signal
Invasive	Involves the introduction of instruments or other objects into the body
Non-invasive	A procedure that does not require inserting an instrument through skin or other body openings
Acute malaria	A form of malaria that may be intermittent or remittent, consisting of chills, fever with its attendant general symptoms and terminating in a sweating stage.
Cross-sectional study	Involves looking at data from a population at one specific point in time
Variable	A characteristic that can be measured and that can assume different values
Clinical malaria	An individual with malaria related symptoms, including fever, chills, severe malaise, headache or vomiting at the time of examination or 1-2 prior to the examination.

Chemotherapy	A drug treatment that uses powerful chemicals to kill fast growing cells in your body
Holoendemic	High transmission area in which the prevalence rate of malaria is over 50% most of the year
Endemic	Endemic (Of a disease/condition) regularly found among particular people or in a certain area.
Pyrogenic threshold	Number of malaria parasites required in the circulation to induce fever
Sporozoites	Motile spore-like stage in the life cycle of some parasitic sporozoans.
Gametocytes	The first step of sexual cycle which occurs inside the anopheline vector
Schizont	Multinucleate form of the parasite that develops in hepatic cells from the
sporozoites	Motile spore-like stage in the life cycle of some parasitic sporozoans that is the ineffective agent introduced into the host.
Half-life	Time taken for concentration of a biological substance to decrease from maximum concentration to half.
Monoclonal	Having a single epitope on the surface
Asymptomatic	Producing or showing symptoms
Submicroscopic	Too small to be seen by an ordinary light microscope
Severe malaria	Occurs when infections are complicated by serious organ failures in the patient’s blood or metabolism

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CHAPTER ONE

INTRODUCTION

1.1 Background

In most parts of the world, particularly in the developing nations of Sub-Saharan Africa, Malaria has been a major problem (Nambati et al 2018). It has posed as heavy economic burden and death majorly in young children under the age of five years and expecting mothers (WHO., 2016). For instance, *P.falciparum* caused a majority of the 229 million malaria cases reported in 2019 with the African region accounting for 94% of total malaria cases.(Ye et al., 2020). Several methods have been employed to effectively manage malaria disease, including use of insecticide treated bed nets, spraying of residential areas using insecticides and treatment of an individual with acute malaria infection using artemisinin. However, the most effective chemotherapy relies on the prompt, accurate and effective diagnosis of malaria due to the high malaria burden reported (Sowumni et al 2011).

Diagnosis currently relies on several methods, most of which are subject to certain limitations. Microscopy which measures peripheral parasitemia remains the gold standard for diagnosing malaria disease (Nantavisai, 2014). Notwithstanding, the method requires highly skilled personnel to operate and is less reliable at low parasite levels (Moutacho et al., 2013). Malaria RDTs operate by targeting *Plasmodium* specific proteins within the patient's infected blood (Wanja et al., 2016). The most common *Plasmodium* proteins targeted include *Plasmodium falciparum* Histidine rich protein-2 (*Pf.*HRP-2), Aldolases and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf.*LDH) (Mouatcho et al., 2013). However, both methods require drawing of blood from patients thereby increasing the chances of transmitting blood-borne diseases as well as causing pain to the patient. Non-invasive approaches to detect in non-blood samples such as

saliva therefore needs to be developed to overcome these problems. Saliva has shown to offer a great deal of potential as a non-invasive diagnostic technique. However, best procedures need to be employed when preparing saliva samples. Also, information on the quantities of saliva antigens needs to be worked out before an off-the shelf diagnostic kit is developed. Several studies have been done on saliva and have successfully detected malaria antigens. However, the results obtained have differed from one study to another. Working with malaria-infected children in Accra, Ghana, Wilson et al detected *Pf.*HRP-2 in whole saliva at 43% sensitivity and 53% in plasma whereas specificity of 100% in both plasma and saliva (Wilson et al., 2009). Studies carried out by Gbotosho et al using a blood-based RDT assay to detect *Pf.*LDH reported sensitivities of 77.9% and 96.7% in whole saliva and saliva supernatant respectively (Nantavisai, 2014). A study done in Lagos state revealed that *Pf.*HRP-2 could be measured in blood with a sensitivity of 96.7% and in saliva at 12.7% (Elendu et al., 2014). It is evident from the above findings that there is lack of consistency in the sensitivity of measuring *Pf.*HRP-2 in different parts of the globe. In view of WHO recommendation, an RDT should achieve a sensitivity of >90% in the *Plasmodium falciparum* detection. Also, the sensitivity and specificity in saliva have not been enhanced in the above studies due to limitation of the commercially available kit used which is designed to detect higher levels of *Pf.*HRP-2 and *Pf.*LDH in whole blood than is found in saliva. The present study thus focusses to adopt a more sensitive chemiluminescent ELISA assay to detect the lower levels of the antigens present in saliva.

In addition, Wilson et al established a relationship between *Plasmodium falciparum* HRP-2 levels in plasma and saliva of patients positive and negative for malaria in Ghana (Wilson et al., 2009). In his studies, Wilson recruited about 30 children with confirmed *P. falciparum* malaria. Of this population, 16(53%) and 13 (43%) had *Pf.*HRP-2 detected in their plasma and saliva

respectfully. Most malaria studies conducted this far on saliva are qualitative and have not quantified the antigens in the saliva (Elendu et al., 2014), making it difficult to attribute the low sensitivities found in the studies to quantities of parasite antigens. The only study in which levels of *Pf*HRP-2 was quantitatively measured in the saliva (Fung et al., 2012) also found a good correlation between levels in blood and those in saliva (Fung et al., 2012). Nevertheless, the study was done in a malaria holoendemic area, with small sample size (eight participants) and major antigens such as LDH and Aldolase were also not tested. There is need therefore to carry out a follow up study to validate the findings in a malaria endemic region. Several studies have reported varying levels of both sensitivity and specificity of RDTs in relation to parasite densities. Results of WHO demonstrated that the Performance of malaria RDTs were below the recommended standards and showed variation at different levels of detection of parasite. (Ogunfowokan et al., 2020). The level of detection however increased with increasing parasite density (McMorrow et al., 2010). Also, Previous findings have illustrated that parasite density decreases across ages. It is maximum in young children and decreases as age increases. A study done in Guinea revealed that the highest densities of parasitemia was reported in children and decreased with increasing age (Koepfli et al., 2015). The efficiency of detection of malaria antigens using blood-based RDTs in regions of high malaria burden have however been shown to be inferior to recommended standards (Laurent et al., 2010). Samples with high levels of parasitemia are vulnerable to prozone effect. This is a false negative test result due to excess of either antibodies or antigens (Maltha, et al., 2013). Prozone effect occurs when antigens are saturated by high antibody levels, preventing a lattice from forming (Luchavez et al., 2011). This observation is of particular concern for malaria endemic zones since parasite density is usually higher in children than adults (Koepfli et al., 2015). For instance, a longitudinal study in

a village in Senegal where *Plasmodium falciparum* malaria is holoendemic showed that parasitemia level was 2.45 parasites /leukocytes up to the age of 12 months, 2.70 parasites/leukocyte between the age of 12 and 23 months, 2.40 parasites/leukocytes at 10 years of age and 0.5 parasites/leukocyte in adults more than 60 years of age (Rogier et al., 1996). Doolan et al has reported similar studies. (Doolan et al., 2009), In his studies, Doolan showed that clinical malaria is maximum in young children and decreases as age increases (Doolan et al 2009). Taken together, the above observations imply that the efficiency of detection of malaria antigens using RDTs is likely to be lower in children in holoendemic settings compared to adults. It is however not known whether prozone effect can affect measurement of malaria proteins in the saliva of patients in malaria endemic zones. It will be interesting to assess whether the performance of blood based RDT differ by age and antigen. Equally important to document is whether there is any age-wise difference in performance of saliva-based assays in holoendemic regions. The present study quantified and reported different levels of *Pf.*HRP-2 and *Pf.*LDH in plasma of acute malaria patients and compared this to levels in saliva. This protocol was different from previous studies in a number of ways. First, major antigens such as LDH were quantitatively determined. Second, a larger sample size was used. Third, this study was conducted in a high malaria endemic zone, (Chulaimbo Sub- County hospital, Kisumu-Kenya). In addition, the effect of parasite density, clinical malaria and age on the levels of salivary *Pf.*HRP-2 in the previous work by (Fung et al., 2012) was not evaluated. This was majorly due to the smaller sample size obtained; hence this stands out to be a major focus in this study. Knowledge on the availability of *Pf.*HRP-2 and *Pf*LDH as biomarkers present in saliva will provide valuable information especially when developing Rapid Diagnostic Test for saliva. Greater signal to noise ratio and smaller detection range are required for an assay that is

acceptable for saliva. This study thus adopted a more sensitive custom chemiluminescent ELISA for *Pf.*HRP-2 and *Pf.*LDH as from a previous study by (Fung et al 2012). Amplification of the signal was achieved using biotinylated detector antibody and the potent tetravalent streptavidin - enzyme combination was used to amplify the signal.

1.2 Statement of the problem

Several studies done on saliva have successfully detected malaria antigen levels in blood and saliva. However, there is no consistency in the studies done in different parts of the world hence the need for follow up studies especially in sub-Saharan Africa, a malaria endemic zone.

Correlation between the levels of malaria antigens in blood and saliva remain unresolved. The only existing study that has examined the malaria antigen *Pf*HRP-2 quantitatively was in Philippines. But the study was carried in a low malaria burden setting, sample size was low (eight participants) and major antigens such as *Pf.*LDH and Aldolase were also not tested. Gaps in knowledge exist on the levels of malaria antigens in the saliva in malaria holoendemic settings, how levels of these antigens in saliva compare with those in the blood, and how these correlates with parasite density.

It is established that the efficiency of detection of malaria based RDTs in malaria endemic regions is below the recommended WHO standards, due to the prozone effect. It is also known that threshold parasitemia for clinical malaria differ with age and parasite density in endemic regions. The implication is that malaria based RDTs should perform differently across different ages, but this has not been determined. Equally unstudied is whether there is any age-wise difference in performance of saliva-based assays in holoendemic settings. It has also not been established whether prozone effect can be observed using saliva media to detect malaria infection. The purpose of this study therefore is to investigate performance and efficiency of

detection of *P. falciparum* antigens (*Pf.*HRP2 and *Pf.*LDH) levels in saliva and blood as biomarkers in the diagnosis of malaria infection using blood-based RDT and saliva-based ELISA assay across different ages.

1.3 Study Objectives

1.3.1 General Objective

To determine the relationship between *P. falciparum* antigens (*Pf.*HRP2 and *Pf.*LDH) levels in saliva and blood as biomarkers in the diagnosis of malaria infection using blood-based RDT and saliva-based ELISA assay across different ages at Chulaimbo-subcounty hospital, Kisumu Kenya.

1.3.2 Specific Objectives

1. To determine the effect of parasite density, clinical malaria and age on the levels of *Pf.*HRP-2 and *Pf.*LDH in saliva and blood of individuals with acute malaria infection at Chulaimbo-subcounty hospital, Kisumu Kenya.

2. To determine the correlation between the levels of *P. falciparum* antigens (*Pf.*HRP-2 and *Pf.*LDH) in saliva and blood of children and adults with acute malaria at Chulaimbo-subcounty hospital, Kisumu Kenya.

3. To compare the sensitivity, specificity, positive and negative predictive values for detection of *P. falciparum* antigens (*Pf.*HRP-2 and *Pf.*LDH) using blood-based rapid diagnostic tests and saliva-based Elisa assay against the gold standard microscopy for diagnosis of malaria infection across different ages at Chulaimbo-subcounty hospital, Kisumu Kenya.

1.3.3.Null hypothesis

1. Parasite density, clinical malaria and age do not predict levels of *Pf.*HRP-2 and *Pf.*LDH in saliva and blood of individuals with acute malaria infection at Chulaimbo-subcounty hospital, Kisumu Kenya.

2. There is no correlation between the levels of P. falciparum antigens (*Pf.*HRP-2 and *Pf.*LDH) in saliva and blood of children and adults with acute malaria at Chulaimbo-subcounty hospital, Kisumu Kenya.

3.There is no significant difference in the sensitivity, specificity, positive and negative predictive values for detection of *P. falciparum* antigens (*Pf.*HRP-2 and *Pf.*LDH) using blood-based rapid diagnostic tests and saliva-based Elisa assay against the gold standard microscopy for diagnosis of malaria infection across different age at Chulaimbo-subcounty hospital, Kisumu Kenya.

1.4 Justification of the Study

Malaria remains a major health hazard leading death and economic burden. About 229 million cases have been reported globally(Ye et al., 2020). Most deaths(92%) have been estimated to have occurred in the African Region. (WHO, 2016). Diagnosis currently relies on microscopy and blood-based Rapid Diagnostic Tests (RDT). However, both methods are invasive tests requiring drawing of blood of patients, thereby increasing risk of accidental infection through needle stick (Maltha et al., 2013).There is thus an urgent need to improve the existing diagnostic tools. Additionally, because microscopy primarily evaluates peripheral parasitemia, it may be a poor predictor of the entire biomass of parasites. Therefore, techniques to assess circulating *Pf.*HRP-2 and *Pf.*.LDH in saliva must be developed in order to enhance predictions of the overall parasite burden especially in malaria endemic setting, preventing death and economic burden associated with malaria. In view of the above public health burden posed by malaria and the

challenges of acute malaria diagnosis, the present study investigated the performance of *P. falciparum* antigens (*Pf*. HRP2 and *Pf*.LDH) levels in saliva as biomarkers for acute malaria in children and adults. It is envisioned that Methods to measure circulating *Pf*.HRP-2 and *Pf*.LDH in saliva would enhance predictions of the overall parasite burden when they are developed, especially in malaria endemic setting, preventing death and economic burden associated with malaria in Africa.

1.5 Significance of the Study

The findings of this study will inform the public health policy makers on better non-invasive malaria diagnostic methods. Measurement of the levels of Pf.HRP-2 AND Pf.LDH if routinely undertaken, may provide the clinician/researcher with a more robust and real time data set to potentially improve diagnosis and focus treatment management. These results will further inform design rules for developing rapid diagnostic test for saliva. The results will also enhance Molecular biologists understanding on the biomarkers for malaria in saliva since quantitation of malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off- the- shelf diagnostic kits. If the burdens of training and instrumentation can be alleviated with automated, portable and sensitive assays, the use of saliva will enable a cost-effective approach for the screening of large populations to enable eradication programs to shift from passive to active surveillance and case management.

1.6 Limitations of the Study

The current study had two main limitations. It was difficult to establish when malaria infection occurred since the study relied on participant's ability to recall time of infection which may not be accurate. It was thus difficult to distinguish between an active and previous infection due to persistence of *Pf*.HRP-2 antigen in circulation. Participants were thus encouraged to recall as accurate as possible the exact time that they started experiencing malaria symptoms. It was also difficult to obtain follow up data especially from adult participants mostly due to their busy schedules. These individuals were encouraged to report back the clinic one month post recovery by explaining to them the benefits of the results of this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria Epidemiology

Malaria is a protozoan disease caused by parasites of the genus *Plasmodium.* Five species of *Plasmodium* are responsible for causing malaria: *P. falciparum, P. vivax, P. ovale, P. malariae*, and *P.knowlesi* (Tangpukdee et al., 2009). Of these, *Plasmodium falciparum* is the most fatal, especially in children under the age of 5 years and pregnant women in Sub-Saharan Africa (WHO, 2016). For instance, *P. falciparum* was responsible for a vast majority of the 212 million cases and more than 400 thousand malaria-related deaths were reported globally in 2017 (Ilesanmi et al., 2017).

2.2 Plasmodium Life Cycle

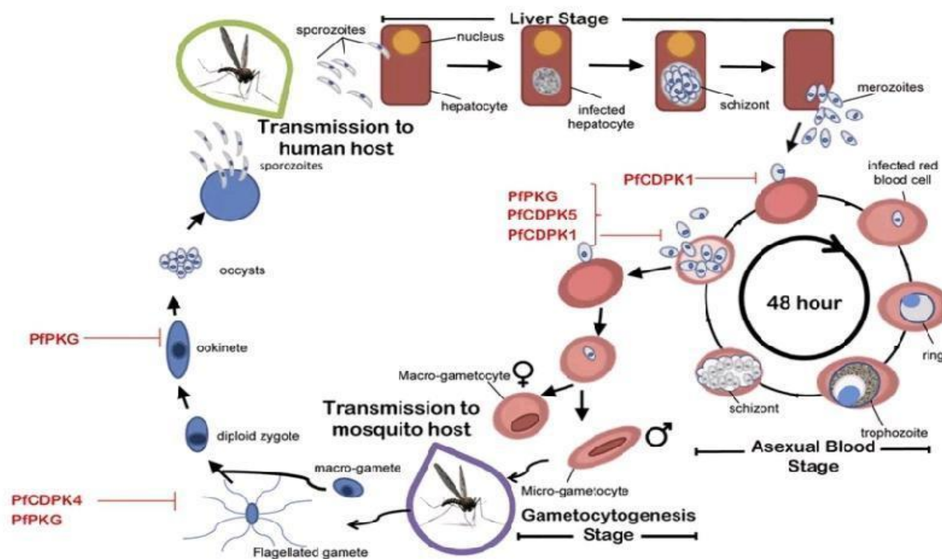

Figure 2.2: Diagram showing the life cycle of *Plasmodium falciparum* (Maltha et al., 2013))

Plasmodium has two types of life cycle. They are sexual and asexual cycle within the mosquito and man respectively (Maltha et al., 2013). Plasmodium sporozoites are injected in the dermis during the bite of an infected female mosquito. They eventually migrate into the circulation and then into liver cells where they grow into schizonts. Approximately one week after infection, schizont rupture releasing merozoites into circulation. Each of the merozoite will enter a red blood cell (RBC) and will develop into trophozoites. Trophozoites will later mature forming schizonts which will later form merozoites. Merozoites will invade new RBCs. Rapture of schizonts and the release of merozoites give rise to the so-called malaria paroxysm with spiking fevers (Maltha et al., 2013). Gametocytes, Plasmodium sexual forms will be produced after one to two weeks. The gametocytes will cause the cycle of transmission to continue back to the mosquito. The male and female gametocytes will within the mosquito forming diploid zygote, which becomes ookinetes (Maltha et al.,2013). Ookinetes will then migrate to the midgut of the insect through the gut wall to form oocytes. Meiotic division of the oocytes and sporozoites will be formed which will migrate to the salivary glands of the female anopheles' mosquito ready to continue the cycle of transmission. These gametocytes will continue the sexual cycle when taken up by a mosquito during a next blood meal. The sexual cycle will be completed over a period of two weeks, whereafter the mosquito can infect another human host (Maltha et al., 2013)

2.3 Malaria Diagnostic Tools

Microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR) are the most currently available malaria diagnostic methods. Microscopy which measures peripheral parasitemia remains the gold. However, the method is not capable of measuring low density parasitemia. It is also an expensive method especially in resource limited countries of Sub-Saharan Africa. Rapid Diagnostic Test methods (RDTs) are more sensitive than microscopy although they only offer qualitative result. On the other hand, molecular techniques such as PCR

are highly sensitive and can detect very low density parasitemia. Although highly sensitive, PCR is expensive. It is also difficult to maintain hence not applicable in many developing countries (Mfuh et al., 2017)

The most extensively used malaria diagnosis tool is microscopy (Xiaodong et al., 2013). It measures asexual parasites. It is used in the examination of blood smear stained with giemsa (Whitty et al., 2008). This technique is sensitive when used by skilled and careful technicians. It is also informative and relatively inexpensive. In addition, microscopy can provide a permanent record of the diagnostic findings and is subject to quality control (World Health Organization, 2000). Microscopy is also very accurate and specific and provides both qualitative (Plasmodium specie) and quantitative (parasite density) data (WHO., 2000). It also reveals the stage of a malaria infection (Xiaodong et al., 2013). It is thus the most commonly used method especially in developing countries. However, shortage of well-trained technologists in most healthcare facilities especially in developing countries may not provide correct data interpretation. This may lead into misdiagnosis leading to death of the patient (Wilson et al 2009). Microscopy is also associated with blood taboos and high risk of infection transmission due to the prick of a needle. The method does not provide immediate results and may not be able to detect the high number of sequestered parasites. The lack of power supply, poor quality microscopes and lack of reagents especially in most developing countries may also prove to be a major challenge (Sowumni et al 2011). Microscopy is also associated with blood taboos. The method only measures peripheral parasitemia leaving behind a high number of sequestered parasites (Chan et al., 2014).

Rapid diagnostic tests (RDTs) represent an excellent opportunity for rapid and precise diagnosis of malaria leading to timely and proper treatment (Nambati et al., 2018). Three types of antigens are targeted: histidine-rich protein 2 (HRP-2, found in *Plasmodium falciparum* only), parasite

lactate dehydrogenase (*Pf*.LDH), and aldolase (pan-malarial antigen, found in all malarial species (Elendu et al., 2017)

RDTs are immunochromatographic tests that provides results within a very short period of time. They can be used as alternative diagnostic tool to microscopy (Nambati et al., 2018). Majorly three types of antigens are targeted, *Pf*.HRP-2, *Pf*.LDH and Aldolase (Elendu et al., 2017). RDTs have several advantages over microscopy. They provide results within a short period of time (15-20) minutes and have a high sensitivity and specificity compared to microscopy (Kiemde et al., 2018). They also offer correct interpretation of data compared to microscopy and are able to detect low density parasites (McMorrow et al., 2010). RDTs are relatively simple and cheap methods for malaria diagnosis. They do not require special equipment to operate or trained personnel (Bendezu et al., 2010). RDTs also provide immediate results and are able to diagnose malaria in regions where individuals are not able to access microscopy. This as a result has lessened disease burden and death in resource-poor malaria endemic settings. However, RDTs give qualitative results only and are unable to quantify parasite density. They cannot differentiate between past and present infection (Oyibo et al 2014). Mutation in the gene encoding the antigen can also affect the results (Oyibo et al., 2014). RDTs are also subject to prozone effect, A false negative test result due to excess of antigens or antibodies (Maltha et al., 2013). False negative results in particular have the potential of harming patient health and damaging the credibility of malaria control programs (Luchavez et al., 2011). Also, failure to detect a case of malaria could lead a clinician to withhold potentially life-saving antimalarial therapy that would have been dispensed if non-specific symptoms based diagnosis had been used (Luchavez et al., 2011). There are also cross-reactions between plasmodium antigens and detection antibodies (Luchavez et al., 2011).

2.4 Rapid Diagnostic Test Target Proteins

Plasmodium falciparum Histidine-Rich Protein-2 (*Pf.*HRP-2) is produced by trophozoites and gametocytes of *Plasmodium falciparum.* It is located in the cytoplasm of the parasite or food vacuole (Dondorp, et al., 2005). It has a molecular weight of 60-105kD and is soluble in water. *Pf.*HRP-2 has a highly unique primary structure. It is made up of histidine which makes up more than 30% of its primary sequence. Its structure majorly comprises of AHHAHHAAD and AHHAAD repeat motifs (Markwalter et al., 2018). The protein has a cleavable sequence which is located at the N-terminus. This plays an active role in its export into host plasma. This allows for the detection of the protein in peripheral circulation (Markwalter et al 2018). Due to its unique structure, *Pf.*HRP-2 is an important biomarker for malaria detection (Kifude et al., 2008) The protein plays an active role in the polymerization of the toxic haem that is produced as a result of degradation of host hemoglobin. This results into the formation of hemozoin, a malaria pigment that is no longer toxic (Mouatcho et al., 2013). *Pf*HRP-2 is expressed in gametocyte and all erythrocytic stages of *P. falciparum* (Mouatcho et al 2013*).* Despite the prolonged half-life of the protein (Poti et al., 2020), *Pf.*HRP-2 could be used to indicate the magnitude of a recent infection.

*Pf.*LDH is the last enzyme in the parasite glycolytic pathway. It is conserved in all human species with malaria hence serves as an active indicator of infection (Jang et al., 2018). Both the sexual and asexual form of the parasite produce the soluble protein (Moutacho et al., 2013). *Pf.*LDH is an essential enzyme in the production of energy since the parasite and the host erythrocyte do not have a complete citric acid cycle for the production of ATP (Moutacho et al., 2013). *Pf.*LDH is produced by the sexual and the asexual parasite form. The protein does not exist in the blood stream once the parasite has been cleared. It is thus useful in tracking parasite

response to treatment and forecasting failure (Gatton et al., 2015). Since the production of *Pf*.LDH are related to parasite viability, the rapid disappearance of *Pf*.LDH in blood after treatment may be due to parasite death following adequate treatment (Houze et al 2009) However, the sensitivity of *Pf*.LDH is lower than that of *Pf*.HRP-2. The parasite Pf.LDH is a tetramer where each monomer consists of two domain LDH folds (Priyamvada et al., 2012). The larger domain comprises the Rossmann fold that binds the co-factor NADH, while the catalytic residues are located in the other domain. These residues are conserved across all plasmodium speciesexcep P.Knowlesi which, which lacks His 195 (Priyamvada et al., 2012).

Aldolase is another glycolytic enzyme found in numerous tissues in the host and in the malaria parasite, where it catalyzes the formation of dihydroxyacetone phosphate and glyceraldehyde-3 phosphate from fructose 1,6-bisphosphate (Mouatcho et al., 2013). Combining Pf.HRP-2 with pan-specific monoclonal antibodies made against Plasmodium aldolase has allowed testing to distinguish between P. falciparum infections and infections caused by other parasites.(Ly et al., 2010). However, few studies have determined the use of antibodies against aldolase for malaria RDTs compared with *Pf*HRP2- and *Pf*.LDH-based tests.

2.5 *Pf*.HRP-2 and *Pf*.LDH as Biomarkers in Blood and Saliva

Microscopy only measures peripheral parasitemia leaving behind a high number of sequestered parasites in the host cytoplasm (Dondorp et al., 2005). While most malaria parasites in the human host replicate asexually, a small proportion of them enter the sexual pathway forming morphologically and functionally different gametocytes (Koepfli et al., 2015). Malaria transmission depends on mosquitoes taking up both male and female gametocyte and transmitting their progeny to the next human host (Koepfli et al., 2015). As the amount of blood

examined is limited, only a small fraction of all parasites are gametocytes. Microscopic detection of gametocytes has limited sensitivity of around (10-20) gametocytes/µL of blood (Koepfli et al., 2015). These parasites effectively adhere to the endothelial lining of microcirculation vessels, which prevents detection of these parasites in peripheral blood film (Goncalves et al.,2014). Both the circulating and the sequestered form of the parasite produce *Pf.*HRP-2 and *Pf.*LDH (Hendriksen et al., 2013). Both plasma and saliva concentrations of these proteins may be used to estimate patient's total parasite biomass (Hendriksen et al., 2013). *P.falciparum* infections can also remain undetectable during pregnancy (Vendrell et al., 2020). The current study quantified plasma and saliva *Pf.*HRP-2 and *Pf.*LDH concentration and determined a relationship between plasma and saliva levels of these proteins and parasite density calculated by microscopy.

2.6 Saliva as a Non-invasive Tool for Malaria Detection and Surveillance

Although blood-based test has several advantages, they are invasive and requires trained personnel (Elendu et al., 2014). They are also associated with the high risk of transmission of infectious diseases (Apinjoh et al., 2021). It is difficult to convince healthy individuals to comply with frequent and multiple blood drawing, children in particular and people with cultural issues (Apinjo et al., 2021). Oral fluids on the other hand are non-invasive, simple and painless (Fung et al., 2012). They can be collected by individuals with very little experience in comparatively larger volumes than blood (Fung et al 2012). Also, no special equipment is needed for the collection of the fluid. Saliva is thus an excellent alternative to blood for both malaria detection and surveillance (Visanuvimol et al., 2015). The use of non-invasive sample such as saliva is therefore increasingly being studied as an alternative source of detection of *P. falciparum*. In fact, saliva has already been used in the identification of numerous immunoglobulins, hormones, medication levels and electrolytes (Sutherland et al.., 2009). Also, saliva has been used

effectively in the detection of the 2019 novel Corona virus, SARS-CoV-2 (Apinjo et al 2021). Additionally, saliva-based assays have been utilized in (HIV) infection diagnosis, forensic medicine, monitoring drug usage, monitoring kidney disorders, preventing cardio-metabolic risk, and detecting and quantifying viral nucleic acids (Alessandro et al., 2015). The release of malaria parasite antigen into the saliva is thought to be associated with fever that leads to vasodilation of vessels supplying the buccal cavity and gingivitis (Fung et al.,2012). Serum molecules can reach saliva through the gingival crevicular fluid and via mechanisms of intracellular and extracellular transport (Fung et al 2012. Numerous studies carried out to detect malaria antigens in saliva have reported mixed findings. In a study to detect *Pf*.HRP-2 in malaria infected children in Accra Ghana, Wilson et al reported a sensitivity of 43% in whole saliva (Wilson et al., 2009). Gbotosho et al also detected Pf.LDH at 77.9 sensitivity in whole saliva and 48% in saliva supernatant (Nantavisai et al., 2014). However, the efficiency of detection of malaria antigens using blood-based RDTs was lower in saliva than blood in both studies. These qualitative investigations indicate the potential of saliva-based malaria assays and also highlight the need for more sensitive tests to quantify the range of Pf.HRP-2 and *Pf.*LDH in saliva. Also a study done in the University of Lagos state, Nigeria, using malaria RDT demonstrated a low sensitivity of 9.4% in saliva and a sensitivity of 96.7% in blood samples (Elendu., 2014). A study done in Philippines using an improved ELISA-based assay that stabilized *Pf.*HRP-2 with a protease inhibitor found 100% sensitivity and specificity in malaria infected adults (Fung et al 2012). It is evident from the above findings that there is inconsistency of measuring Pf.HRP-2 and Pf.LDH in studies done in these different parts of the world. There is need for follow up studies especially in Sub-Saharan Africa.

2.7 Correlation Between the Levels of P. falciparum Antigens (Pf.HRP-2 and Pf.LDH) in Saliva and Blood of Children and Adults with Acute Malaria infection.

P. falciparum Histidine rich protein2 (*Pf.*HRP-2) and *Plasmodium* specific Lactate dehydrogenase (*Pf.*LDH) are parasite enzymes released into the host blood during clinical malaria infection, and have been demonstrated in oral fluid. In a study to detect *Pf.*HRP-2, in whole saliva, Wilson et al reported sensitivity of 43% (Fung et l., 2012). On the other hand, Gbotosho reported sensitivity of 77.9% and 48.4% in whole saliva and saliva supernatant respectively (Fung et al., 2012). Both results revealed that the efficiency of detection was lower in saliva than plasma. There is need to quantitatively asses the levels of these antigens in blood and saliva and determine their relationship with parasite density. Studies carried out at Msambweni district hospital, Kenya revealed a weak positive correlation in the levels of *Pf.*LDH in saliva and blood (Nambati et al., 2018). *Pf.*LDH levels were lower in saliva than plasma of individuals with similar levels of parasitemia. A study that quantitatively measured *Plasmodium falciparum* antigen levels in saliva and blood of eight patients positive for malaria in the Philippines found a 100% sensitivity and specificity in both plasma and saliva of individuals (Fung et al 2012). A study done in Lagos state using 1026 sample patients revealed that the test results of individuals varied in blood and saliva (Elendu et al., 2014). Among the 181(18.1%) of patients that were positive for malaria, 24(12.9) % of the saliva samples were also found to be positive (Elendu et al., 2014).

Most malaria studies conducted this far on saliva have not quantified the antigens in the saliva (Nantavisai et al., 2014). This makes it difficult to attribute the low sensitivities found in the studies to quantities of parasite antigens. The only one study in which levels of *Pf*HRP-2 was quantitatively measured in the saliva (Fung et al., 2012b) and found a good correlation between

levels in blood and those in saliva experienced a few limitations. First, the study was conducted in a low malaria setting and sample size was low (eight participants). Second, major antigens such as *Pf.*LDH and Aldolase were also not tested. There is need therefore to carry out a follow up study to validate these findings in a malaria endemic region. This study partly aims at quantitatively determining the levels of malaria antigens in both saliva and blood so as to correlate this to parasite densities. Information on the correlation between the levels in the blood and saliva will provide insight as to whether one can be substituted for another.

2.8 Malaria Endemicity and RDTs

Several studies have reported varying levels of both sensitivity and specificity of RDTs in relation to parasite densities. Findings of WHO observed that Performance of malaria RDTs were relatively low and increased with increasing parasite density (Mcmorrow et al., 2010). This was clearly demonstrated in a study carried out in Tanzania to detect *Pf.*HRP-2. These tests demonstrated sensitivity and specificity above 90% at parasite density above 2000/µL of blood. Sensitivity of RDTs however reduced to 50% when detecting lower density parasite (6-50)/µL (McMorrow et al., 2010). Findings have also illustarted that parasite density is higher in children than in adults (Lawrence et al., 1995). In contrast, studies in Papua New Gunea illustrated that the prevalence of *Pf.*HRP-2 was highest in children but decreased in adults (Koepfli et al., 2015). However the performance of Rapid Diagnostic Tests (RDTs) in areas of high malaria burden have been shown to be inferior to recommended standards due to prozone effect (Laurent et al., 2010). Prozone effect refers to a false negative results in immunological reactions brought on by overabundance of either antigens or antibodies. High antibody concentrations can overwhelm antigens causing a prozone effect, which hinders lattice formation and precipitation (Luchivez et

al.,2011). This observation is of particular concern for malaria endemic zones since parasitemia is usually high in the younger ages. For instance, a longitudinal study in a village in Senegal where *Plasmodium falciparum* malaria is holoendemic showed that the threshold parasitemia level was higher in children than in adults (Lawrence et al.,1995). Similar studies reported by Doolan et al have revealed that the risk of clinical malaria increases from birth and is maximum in individuals below the age of 5 years above which the risk of clinical malaria sharply decreases (Doolan et al., 2009). Findings in South west Nigeria also demonstrated that parasite prevalence was higher in children than adults (Afolabi et al., 2016).

Taken together, the above observations have established that the efficiency of detection of malaria antigens using RDTs is higher in children in holoendemic settings than adults. It will thus be interesting to assess whether the performance of blood-based RDT differ by age and antigen. Equally important to document is whether there is any age wise difference in the performance of saliva-based assay in holoendemic setting.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This study was carried out in Chulaimbo sub-county hospital. Chulaimbo is a malaria endemic region where residents receive about 100-300 ineffective mosquito bites per annum and *P. falciparum* accounts for 97% of malaria infections. Chulaimbo sub-county hospital is a government health center located in Marera sub-location North West Kisumu, Maseno Division as shown in Appendix III.

3.2. Study Design

3.2.1 Research Design

The above research project adopted a cross-sectional study design.

3.2.2. Sample Size Determination

The Fishers formula below was used to calculate the total sample size for both children and adults required to participate in the study (Sin-Ho Jung et al., 2014).

$$n \geq \frac{Z^2\alpha/_2 P(1-P)}{d^2}$$

Where;

- n = Minimum sample size of people confirmed with acute malaria infection
- $Z^2\alpha/_2$= Standard normal distribution critical value at α-level of significance (α=0.05, $Z^2\alpha/_{2=1.96}$)
- P_2 = Estimated proportion of population with characteristics of interest(p=0.72)
- d= Desired marginal error d (0.1)

- $\frac{1.96^2 x 0.72(1-0.72)}{0.1^2}$

Therefore, the minimum sample size required for this study was n=78 patients

3.3.3. Inclusion Criteria

1. Individuals at least six months and above were incorporated into the study.

2. Individuals with microscopically confirmed *P. falciparum* infection.

3. Individuals who were able to give informed consent from parent/guardian to participate in the study.

3.3.4. Exclusion Criteria

1. Individuals who showed symptoms for other forms of illness apart from malaria, however no individual met these criteria for exclusion

2. Persons with severe malaria, however, no such cases were found

3.4. Study Population.

This study recruited participants with confirmed *P. falciparum* infection at Chulaimbo sub-county hospital in Kisumu West Sub- County Kenya. Kisumu is in a malarial endemic area, with intense year-round transmission of malaria. It majorly targeted children and adults. It has been established that the efficiency of detection of blood-based RDTs is dependent on parasite density which in turn decreases with age. This study aimed to determine whether the performance of blood-based RTDs and saliva-based ELISA assay differed across ages. Matched samples of blood and saliva were collected from each patient upon recruitment and processed at Chulaimbo

sub-county hospital after ethical approval by Maseno University Ethical Review Committee (MUERC). It was anticipated that participants would gain future benefits should there be interventions or policy changes as a result of the research project.

A total of 74 children and 20 adults had their blood and saliva samples collected for quantitative determination of *P. falciparum* antigens. Repeat negative control samples were obtained from the same patients 4 weeks post recovery where samples from a total of 33 participants mostly consisting of children were obtained.

3.5. Blood Sample Collection

Capillary tubes and pre-cleaned frosted-end slides were first labelled with the patient's name, date and time of collection. After explaining the procedure to the participants, the middle fingertip was cleaned using alcohol swab and a standard prick made to enable blood flow. The first drop of blood was wiped with a clean gauze followed by gently squeezing finger. Using a capillary tube, approximately 1mL of blood was collected from each patient by trained phlebotomist. Information on date of collection, date of birth, auxiliary temperatures, body weights and whether participants were on antimalarial treatment or not were also recorded in an excel sheet document.

3.6. Microscopic Diagnosis of Malaria

Soon after a finger prick, thick and thin films were immediately prepared on the same slide. To prepare thin film, a clean spreader slide held at a 45° angle was brought towards the drop of blood on the specimen While sliding it at the same angle and pushing it forward rapidly and smoothly. Approximately 2μL drop of blood was used for thin smear. For thick film,

approximately 6µL drop of blood was spread using the corner of a clean slide in a circle the size of a dime (diameter 1-2 cm). Care was taken not to make the smear too thick or it would have fallen off the slide. Thin film was fixed with methanol (absolute methanol) and allowed to dry completely before staining. Thick film was not fixed. Both films were left to completely dry before staining. 10% giemsa stain solution was first prepared in a coupling jar and the thick film dehemoglobinized by immersing in water. Both the thick and thin film were flooded in with working giemsa stain for 20 minutes. The slides were then flooded with water to remove the excess stain. After 30 minutes the slides were allowed to air-dry in a vertical position and examined under a light microscope. 100x oil immersion objective was used in the study. Parasites were counted against 200wbcs and their density calculated with reference to 8,000 white blood cells/µl. Blood slide were considered negative for plasmodium species if no parasites were visible.

3.7 Measurement of Hemoglobin levels

A drop of venous blood was introduced into the microcuvette by capillary action, and after reaction with the reagents, the absorbance was read in the hemocue photometer at 565 and 880nm. The hemoglobin concentration was then displayed as a digital reading in g/dl.

3.8. Malaria Rapid Diagnostic Test

Malaria diagnosis by commercial RDTs were performed on all blood samples following the manufacturer's instruction. Using capillary tubes, approximately 20 µl of blood finger prick blood sample was transferred to the test cassette and about 3 drops of buffer added to the buffer well. In this method, the liquid specimen was applied to one end of the strip where it mixed with lysing agents, buffer and labeled antibody. The fluid mixture then migrated across the

nitrocellulose membrane where it was captured by fixed antibodies and the results read after 15 minutes.

3.9. Saliva Collection and Processing

Saliva samples were collected from each participant with confirmed *P.falciparum* as previously described (Fung et al., 2012). About 3ml saliva was collected from each participant positive for malaria. Participants expectorated into 5ml saliva collecting tubes. The saliva sample was immediately labeled and placed in a cool box and transported to Kemri-Kisian Laboratory within 3 hours for processing. In the laboratory, Saliva samples were centrifuged at 2600 rpm and 4^0C for 15 minutes and Supernatant aliquoted into two equal portions. The supernatant was then processed by adding the following; Aprotinin (0.9µl, 6 .12 Units/mL), Na_3OV_4 (3µ L at 400 mM, pH10 in water), and PMSF (10µL at 10mg/mL in isopropyl alcohol was added per 1mL supernatant. Samples were stored at -20^0C until laboratory analysis. Prior to analysis, the samples were thawed, centrifuged and separated again, and 5% v/v 20× PBS-T was added to the supernatant.

3.9.1 An Antibody Sandwich ELISA to Detect Soluble Pf. HRP2 and Pf.LDH in Plasma /saliva Supernatant.

Microtiter plates were coated with 50μL/well of diluted capture antibody in coating buffer (PBS). The plates were incubated for overnight at 4 °C. The wells were emptied and the plates washed 3x times with PBS-T wash solution. The remaining drops were removed by patting the plates on an absorbent paper after final wash. Blocking was achieved by adding 50μL/well blocking solution (PBS-T containing 4 wt.% dry milk powder). Plates were incubated for 1 hour at room temperature. The plates were emptied and washed 3x with wash solution (PBS-T). Blank

sample and eight calibration solutions (standards) of recombinant antigens of concentration ranging between (0.05-5.0) ng/uL of HRP-2 and ($132-1.32^{-8}$) ng/uL of LDH were prepared.

Standards, controls and blank were run on the same plate as sample to ensure accuracy. 200μL of the antigen test solution, standard antigen dilutions, ==-blanks and controls were added to the antibody coated wells. The plates were covered with adhesive plastic film and incubated for 1 hour at room temperature. The plates were emptied and the wells washed 5X times with PBS-T. 50uL/well of diluted biotinylated detection antibody was then added to the wells. The plates were covered with adhesive plastic film and incubated for 1 hour at room temperature. The plates were emptied and washed 5x with PBS-T solution. 50μL per well of peroxidase-labelled streptavidin (0.8 μg/mL in blocking buffer: 4µL of stock solution(1mg/ml) was added into 5mL buffer for every plate). The plates were incubated for 30 min at room temperature under agitation. Plates were emptied and washed 5x times in PBS-T. 50μL per well of chemiluminescent substrate was added and the plates incubated at room temperature for 25 min. Luminescence signal was read immediately at (460/20) RLU.

3.9.2 Data Management and Analysis

Levels of *P. falciparum* antigens in saliva and blood was measured using ELISA. Exploratory analysis was done to describe the data: Measures of central tendency and dispersion: Mean (SD), Median (Range) values were reported for quantitative variables like age of patients, auxiliary temperature, hemoglobin levels and parasite density. Generalized linear model (GLM) was done to assess the relationship between the levels of *P. falciparum* antigens to parasite densities, clinical malaria and age. Correlation between levels of *Pf.* HRP-2 and *Pf.* LDH antigens in blood and saliva were evaluated using Pearson correlation. Sensitivity, specificity, positive and

negative predictive values of the blood-based rapid diagnostic tests and saliva-based Elisa assay was calculated in both children and adults.

NB: To measure sensitivity, specificity, positive and negative predictive values, microscopy acted as the gold standard. These values were expressed as percentage.

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True positive + False Negative}} \times 100\%$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative + False positive}} \times 100\%$$

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True Positive + False positive}} \times 100\%$$

$$\text{Negative predictive value} = \frac{\text{True Negative}}{\text{True Negative + False Negative}} \times 100\%$$

CHAPTER FOUR

RESULTS

Table 4.1.1 Data collected from malaria- positive individuals and their controls

Sample ID	Gender	Blood smear results	If *Pf*. Positive	RDT results	HRP-2 in plasma(ng/mL)	HRP-2 in saliva(ng/mL)	LDH in plasma(ng/mL)	LDH in saliva(ng/mL)
EAM-AC-063	Male	Pos	1335	Pos	62.994	6.0885	2.71530474	0.293847072
EAM-AC-064	Female	Pos	700	Pos	62.5425	77.1045	3.933399994	3.268085012
UM-074	Female	Pos	600	Pos	76.7895	4.365	36.34672815	0.616624019
EAM-AC-065	Male	Pos	660	Pos	47.157	5.6625	No sample	1.874429207
UM-075	Female	Pos	-	Pos	21.558	2.3295	0	0.096665781
EAM-AC-066	Male	Pos	660	Pos	90.4665	1.4475	231.8531484	0.202848515
EAM-AC-067	Female	Pos	800	Pos	131	1.482	4.73415944	0.293847072
EAM-AC068	Male	Pos	600	Pos	54.9915	1.281	23827.70338	20.84687778
EAM	Femal	Pos	1060	Pos	56.8185	0.8235	3.26808501	2.2560244

-AC069	e						2	93
EAM-AC-070	Male	Pos	571	Pos	49.0155	5.4	0.3536683	0.3536683
EAM-AC-071	Female	Pos	460	Pos	12.093	4.5525	7.478328228	0.742156004
EAM-AC-073	Female	Pos	-	Pos	56.811	0.6045	9.93440704	1.362756202
EAM-AC-074	Male	Pos	700	Pos	63.8265	1.6405	1020.965666	0.168537701
EAM-AC-076	Female	Pos	40	Pos	40.6695	0.489	4.73415944	76.27186971
EAM-AC-079	Female	Pos	500		30.213	185.1	0.512325143	0.0010218
EAM-AC-118	Mae	Pos	35	Pos	0	0	0.0067203	0
EAM-AC-119	Male	Pos	112	Pos	0	0	0.0050304	0
EAM-AC-81	Female	Pos	80	Pos	158.9795	1.474	13.80332173	1.458792835
EAM-AC-84	Male	Pos	1100	Pos	151.9795	0.402	8.005343595	3.302927737
EAM-AC-	Female	Pos	500	Pos	198.4875	0.47	8.569498974	1.915558583

85								
EAM-AC-87	Female	Pos	1400	Pos	186.0065	3.634	2.692605381	0.0067203
EAM-AC-104	Female	Pos	120	Pos	43.56	0.0666	0.0060129	4.337114513
EAM-AC-105	Female	Pos	860	Pos	231.7	230.5	0.0035763	4.051589479
EAM-AC-083	Male	Pos	70		405.1	227.5	3.933399994	0.0015327
EAM-AC-101	Female	Pos	2800	Pos	357.3	101.7	0	10.51191387
EAM-AC-082	Male	Pos	105	Pos	33.7565	1.396	63.37086354	0.000786
EAM-AC-086	Male	Pos	1600	Pos	146.2115	0.358	0.616624019	0
EAM-AC-091	Female	Pos	400	Pos	184.939	2.592	3.933399994	0.0040479
EAM-AC-095	Female	Pos	100	Pos	150.9155	4.65	1.075089797	0.0014541
EAM-AC-096	Female	Pos	130	Pos	160.8555	4.65	0.096665781	0.0009825
EAM-AC-097	Female	Pos	200	Pos	129.7265	5.75	23827.70338	0.0009432

EAM-AC099	Female	Pos	480	Pos	163.4945	24.45	0	0.0011397
EAM-AC-100	Female	Pos	400	Pos	136.562	16.75	0	1.18923384
EAM-AC-102	Male	Pos	177	Pos	20.5335	0.4834	0.0127725	29.19534469
EAM-AC-072	Male	Pos	350	Pos	0	9.694	0	52.65199819
EAM-AC-074	Male	Pos	700	Pos	56.811	1.6405	1.020965666	0.168537701
EAM-AC-076	Female	Pos	40	Pos	40.6695	0.489	0.004734159	76.27186971
EAM-AC-077	Male	Pos	100	Pos	-	14.4435	-	0.293847072
EAM-AC-103	Male	Pos	857	Pos	29.932	0.0674	0.0072312	2.195059752
EAM-AC-106	Male	Pos	3000	Pos	54.134	0.0334	0.002358	3.535692818
EAM-AC-107	Male	Pos	100	Pos	21.226	0.0222	0.0016506	1.561597394
EAM-AC-108	Male	Pos	600	Pos	22.142	0.0146	0.0010218	2.692605381
EAM	Male	Pos	35	Pos	31.922	0.0082	0.0011004	2.1950597

-AC-109								52
EAM-AC118	Male	Pos	35	Pos	25.546	0.3746	0.0067203	-
EAM-AC-119	Male	Pos	112	Pos	30.902	0.3058	0.0050304	-
EAM-AC-078	Male	Pos	600	Pos	45.9615	2.394	-	-
EAM-AC-090	Male	Pos	125	Pos	17.3	17.3	-	0.0035763
EAM-RE-080	Male	Neg	0	Pos	0	7.0365	4.051589479	0.0009432
EAM-RE-082	Male		0	Pos	237.6	23.9	2.882359611	0.0117507
EAM-RE-083	Male	Neg	0	Pos	34.8		-	-
EAM-RE-085	Female	Neg	0	Pos	8.8	0	2.349750772	0.001179
EAM-RE-084	Male	Neg	0	Neg	8.8	0.0166	2.692605381	10.51191387
EAM-RE-087	Female	Neg	0	Neg	40	0.1186	1.671646833	-
EAM-RE-	Female	Neg	0	Neg	11.4	0.0886	1.362756202	2.882359611

090								
EAM-RE-091	Female	Neg	0	Pos	24.4	0.0642	2.195059752	4.051589479
EAM-RE-092	Male	Neg	0	Pos	53	0.0682	5.695117724	1.789451714
EAM-RE-093	Male	Pos	320	Pos	237.6	0.0498	6.096466055	2.515343232
EAM-RE-094	Male	Neg	0	Pos	40	0.0666	1.671646833	15.81737893
EAM-RE-095	Female	Neg	0	Pos	-4.2	0.0222	3.085486268	3.784861401
EAM-RE-096		Neg	0	Pos		0.0122		1.110943071
EAM-RE-097	Female	Neg	0	Pos	3.6	0.0094	2.515343232	12.04571829
EAM-RE-63	Male	Neg	0	Neg	282.4		0.0242874	-
EAM-RE-75	Male	Pos	330	Neg	-4.2	104.1	0.0242874	0.0030261
EAM-RE-071	Female	Neg	0	Pos	86.6	1.3255	0.0064845	0.742156004
EAM-RE-092	Male	Neg	0	Pos	53	0.0682	5.695117724	1.789451714
EAM	Male	Neg	0	Pos	40	0.029	1.67164683	15.817378

-RE-094							3	93
EAM-RE-095	Female	Neg	0	Pos	-4.2	0.0414	3.085486268	3.784861401
EAM-RE-076	Female	Neg	0	Neg	113	0.428	0.0027903	1.18923384
EAM-RE-078	Male	Neg	0	Pos	11.4	0.288	1.789451714	0.0120258
EAM-RE-066	Male	Neg	0	Neg	99.8	8.791	-	0.3536683
EAM-RE-068	Male	Neg	0	Pos	236.75	0.8215	0.0015327	0.742156004
EAM-RE-069	Female	Neg	0	Pos	210.35	-	0.001179	-
EAM-RE-070	Male	Neg	0	Neg	187.25	0.8215	0.0007467	0.055443222
EAM-RE-077	Male	Neg	0	pos	251.05	51.4	-	0.0006681
ID-1070-DO	-	-	-	-	23.724	4.2795	0.0049518	1.789451714
ID-1071-DO	-	-	-	-	4.658	4.6374	0.0032226	6.85792001
ID-1072-DO	Male	Pos	15	Pos	8.068	3.8205	0.0022401	9.93440704

ID-1073-DO	Female	Pos	600	Pos	28.006	1.7865	0.001179	0.742156004
ID-1075-DO	Female	Pos	12	Pos	3.428	0.4485	-	0.425667902
ID-1074-DO	Male	Pos	360	Pos	25.064	1.437	0.0010218	20.84687778
ID-1078-DO	Male	Pos	20	Pos	296.9	403.5	14.77607425	0.0011004
ID-1082-DO	Male	Pos	3	Pos	634.4	0.0546	3.784861401	2.692605381
ID-1076-DO	Female	Pos	320	Pos	6.578	9.0675	0.0108468	52.65199819
ID-1077-DO	Male	Pos	400	Pos	30.18	6.2885	0.0067203	0.244144306
ID-1080-DO	Female	Pos	325	Pos	6988.55	0.326	0.000786	0.0010611
ID-1081-DO	Female	Pos	60	Pos	4264.4	14	0.0022401	0.0004323
ID-1082-DO	Male	Pos	3	Pos	634.4	0.0546	3.784861401	2.692605381
ID-1083-DO	Female	Pos	350	Pos	5210.4	0.0434	0.0009432	4.051589479
ID-		Neg	0	Neg	-4.2	7.8355	3.30292773	17.320732

1070-D28							7	43
ID-1071-D28		Neg	0	Neg	16.6	7.657	No sample	0.512325143
ID-1077-D28	Male	Neg	0	Pos	1	51.4	1.037806414	0.0089211
ID-1079-D28		Neg	0	Neg	1	32.7	3.085486268	0.0048732
ID-1082-D28	Male	Neg	0	Neg	-6.8	-	3.784861401	12.89460837
ID-1078-D28	Male	Neg	0	Neg	14	17.85	47.02640574	0.0008253

Analysis of blood and saliva specimen -Microscopy of thick-films smears was the gold standard for diagnosis**.** **EAM-AC** -Acute malaria children, **EAM-RE**- Recovery children, **ID-DO**-Acute malaria adults**,** **ID-D28**-Recovery adults

4.1.3 Demographic and Clinical Information

A total of 74 children and 20 adults were enrolled into the study. At acute, the age of children ranged between (3.3-12.1) years with a median value of 7.1 whereas adults ranged between (19.3-64.1) years with a median value of 26.7 respectively. The mean (SD) hemoglobin levels in children were 11.2(2.1) and 12.9(2.8) in adults. Hemoglobin levels ranged between (5.6-16.2) g/dL with a median value of 11.3g/dL and (7-16.6) g/dL with a median value of 13.9 in adults respectively. These range values reduced slightly one month post treatment with children recording hemoglobin levels ranging between (7.2-14.8) g/dL and a median value of 11.9g/dL

and adults recording hemoglobin values ranging between (14.2-15.5) g/dL and a median value of 15.2g/dL. Overall, the mean parasite density was found to be higher in children compared to adults. At acute, the mean (SD) parasite density was found to be 586.7(640) in children and 222.8(198.3) in adults. These values reduced significantly upon treatment with anti-malarial drugs giving mean (SD) of 325(7.1) in children and zero values in adults, (Table 4.1).

Levels and frequencies of *Plasmodium falciparum* antigens by ELISA were also determined. In children, the mean (SD) Pf.HRP-2 was higher 93.6(99.8) than *Pf.*LDH 38.4(168.2). The frequency of plasma Pf.HRP-2 was higher in acute malaria children 38(70.3%) than in individuals one month post treatment, 16(59.3). In adults, the mean (SD) Pf.HRP-2 levels in both plasma and saliva were higher 1288.2(2348.4) and 32.2(106.9) respectively than *Pf.*LDH 1.4(4.1)and7.2(14.3)respectively(Table4.1.2).

Table 4.1.3 Demographic Information

	Acute Malaria Cases				Recovery Cases			
	Age (years)	**Hb(g/dL)**	**Parasitaemia(/μL)**	**Temp (^{O}C)**	**Age (years)**	**Hb(g/dL)**	**Parasitaemia(/uL)**	**Temp (^{O}C)**
Acute children (n=47)					**Recovery Children(n=27)**			
Mean (SD)	6.9(2.4)	11.2(2.1)	586.7(640)	38.6(0.9)	7.2(2.6)	11.9(1.6)	325(7.1)	36.3(0.3)
Median (Range)	7.1(3.3-12.1)	11.3(5.6-16.2)	500(35-3000)	36.4(37.5-40.5)	26.4(3.4-10.8)	11.9(7.2-14.8)	325(0-330)	36.3(35.8-36.9)
Acute Adults(n=14)					**Recovery Adults(n=6)**			
Mean (SD)	33.6(2.4)	12.9(2.8)	222.1(198.3)	36.7(0.8)	36(20.1)	14.9(0.7)	0(0)	36.2(0.324)
Median (Range)	26.7(19.3-64.1)	13.9(7-16.6)	260(3-600)	36.4(35.8-38.9)	26.4(25.1-66.1)	15.2(14.2-15.5)	0(0)	36.2(35.8-36.7)

Table 4.1.4 Levels and Frequencies of *Plasmodium falciparum* Antigens by ELISA

	Acute Malaria Cases(n=47)		**Recovery Cases(n=27)**	
Children	**Plasma**	**Saliva**	**Plasma**	**Saliva**
*Pf.*HRP-2 levels, Mean (SD)	93.6(99.8)ng/mL	21.2(54.5)ng/mL	87.4(97)ng/mL	8.3(23.3) ng/mL
*Pf.*HRP-*2* frequency, (n (%))	38(70.3%)	5(10.6%)	16(59.3%)	2(8.3%)
*Pf.*LDH 2 levels, Mean (SD) (ng/mL)	38.4(168.2)ng/mL	7.5(18.5)ng/mL	2.1(1.9)ng/mL	3.4(4.9) ng/mL
*Pf.*LDH frequency, (n (%))	6(14%)	3(7.3%)	0(0%)	0(0%)
Adults	**Acute Malaria Cases(n=14)**		**Recovery cases(n=6)**	
*Pf.*HRP-2 levels, Mean (SD), (ng/mL)	1288.2(2348.4)ng/mL	32.2(106.9)ng/mL	5.4(7.7)ng/mL	23.5(18.7)ng/mL
*Pf.*HRP-2 frequency, (n (%))	9(64.3%)	1(7.1)	0(0%)	2(40%)
*Pf.*LDH levels, Mean (SD)	1.4(4.1) ng/mL	7.2(14.3)ng/mL	11.6(17.7) ng/mL	5.1(7.9) ng/mL
*Pf.*LDH frequency, (n(%))	0(0%)	1(7.1%)	0(0%)	0(0%)

SD= Standard Deviation, **n**= Frequency of samples with detectable *Pf.*HRP-2 and *Pf.*LDH levels.

4.2 Relationship between Parasite Density, Clinical Malaria and Age Versus the Levels of Pf.HRP-2 and Pf.LDH in Saliva and Blood of Individuals with Acute Malaria Infection.

To determine whether the levels of *Plasmodium falciparum* Antigens can be a biomarker for Plasmodium infection in blood and saliva, the concentration of *Pf.*HRP-2 and *Pf.*LDH measured by ELISA was compared with blood smear parasite densities, clinical malaria and age. Parasite density did not significantly predict *Pf.*HRP-2 levels in plasma and saliva, (F=0.001, *p*=0.974)) and (F=0.228, *p*=0.635) respectively. Also, parasite density did not significantly predict *Pf.*LDH levels in plasma and saliva, (F=0.327, *p*=0.570) and (F=1.029, *p*=0.315) respectively. The concentration of *Pf.*HRP-2 and *Pf.*LDH was also compared with clinical malaria. Clinical malaria did not significantly predict *Pf.*HRP-2 antigens in both plasma and saliva of individuals with acute malaria infection, (F=1.841, *p*=0.179) and (F=0.017, *p*=0.895) respectively. Equally, clinical malaria did not significantly predict *Pf.*LDH antigens in both plasma and saliva, (F=1.131, *p*=0.291) and (F=1.222, *p*=0.272) respectively. The study also determined if age is a good predictor of the antigen levels in both plasma and saliva. In contrast, age significantly predicted *Pf.*HRP-2 levels in plasma (F=14.329, *p*=0.00) but not in saliva (F=0.310, *p*=0.580). *Pf.*LDH antigens were also analysed using the same model. Age did not significantly predict *Pf.*LDH levels in both plasma and saliva of individuals with acute malaria infection, (F=0.701, *p*=0.406) and (F=0.091, *p*=0.764) respectively, (Table 4.2).

Table 4.2: Relationship Between Parasite density, Clinical malaria and Age and the Levels of *Pf.* HRP-2 and *Pf.* LDH in a Western Kenya Population.

n=47-Children; 14-Adults	**F-statistic**	***p*-value**
Pf. HRP-2 in plasma versus Parasite density	0.001	0.974
Pf. HRP-2 in saliva versus Parasite density	0.228	0.635
Pf. LDH in plasma versus Parasite density	0.327	0.570
Pf. LDH in saliva versus Parasite density	1.029	0.315
Pf. HRP-2 in plasma versus Clinical Malaria	1.841	0.179
Pf. HRP-2 in saliva versus Clinical Malaria	0.017	0.895
Pf. LDH in plasma versus Clinical Malaria	1.131	0.291
Pf. LDH in saliva versus Clinical Malaria	1.222	0.272
Pf. HRP-2 in plasma versus age	14.329	0.000
Pf. HRP-2 in saliva versus age	0.310	0.580
Pf. LDH in plasma versus Age	0.701	0.406
Pf. LDH in saliva versus age	0.091	0.764

Relationship between parasite density, clinical malaria and age versus antigen levels was determined using a generalized linear model.

4.3. Correlation between the Levels of *Pf.* HRP-2 and *Pf.* LDH in Saliva and Blood of Children and Adults with Acute Malaria

To determine whether saliva can be used as an alternative non-invasive diagnostic tool to plasma, the correlation between the levels of *Pf.* HRP-2 and *Pf.* LDH in plasma and saliva of individuals was determined. There was no significant relationship in the levels of *Pf.*HRP-2 in plasma and saliva (r=-0.235, *p*=0.104). However, results indicated a significant relationship in the levels of *Pf.*HRP-2 in both plasma and saliva of individuals one month post treatment

(r=0.685, *p*=0.001). There was no significant relationship in the levels of *Pf.*LDH in plasma and saliva of acute malaria individuals (r=-0.0235, *p*=0.104). Equally, no significant relationship was observed in *Pf.*LDH levels one month post treatment (r=-0.132, *p*=0.547) (Table 4.3)

Table 4.3: Relationship between the Levels of *Pf.*HRP-2 and *Pf.*LDH in Plasma and Saliva of Children and Adults with Acute Malaria in a Western Kenya Population

	Acute Malaria Cases, Children, n=47; Adults, n=14		**Recovery Cases, Children, n=27; Adults, n=6**	
	R	*p*	r	*p*
*Pf.*HRP-2	-0.026	0.850	0.685	0.001
Pf. LDH	-0.063	0.673	-0.130	0.575

Levels of *Pf.*HRP-2 and *Pf.*LDH in plasma and saliva was determined using Pearson correlation

4.4. Efficiency of Detection of (Pf.HRP-2 and Pf.LDH) using Blood-based Rapid Diagnostic Tests

The comparative performance of the plasma assays using blood-based RDTs in both children and adults are shown in table 4.4 below. Using blood-based RDTs, sensitivity of detection was found to be slightly lower in children (98%) than in adults (100%). Specificity was significantly lower in children (28%) than in adults (83%). Positive predictive value was 73% in acute malaria children and 92% in adults. Negative predictive value was 88% in children and 100% in acute adults.

Table 4.4 Sensitivity, Specificity, Positive and Negative Predictive Value for Detection of Malaria Antigens using Blood-Based RDTs in Children and Adults.

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative Predictive value**	**N**
Children	98%	28%	73%	88%	74
Adults	100%	83%	92%	100%	20

Efficiency of detection of *Pf.* Antigens (*Pf.*HRP-2, *Pf.*LDH), Microscopy was used as the gold standard,

4.5 Efficiency of Detection of P. falciparum Antigens (Pf.HRP-2 and Pf.LDH) using ELISA-based Assay in Children and Adults.

Further, Sensitivity and specificity for the detection of *Falciparum* antigens (*Pf.*HRP-2 and *Pf.*LDH) using ELISA was evaluated against microscopy. Sensitivity of *Pf.*HRP-2 was found to be 78% in plasma and 13% in saliva of children. *Pf.*LDH had a sensitivity of 15% in plasma and 7% in the saliva. In adults, *Pf.*HRP-2 had a sensitivity of 75% in plasma and 50% in saliva. Sensitivity of *Pf.*LDH was 0% in plasma and 8.3% in saliva. Specificity of *Pf.*HRP-2 was 40% in plasma and 95% in saliva of children. *Pf.*LDH was 100% in both plasma and saliva. In adults, specificity of *Pf.*HRP-2 was 100% in plasma and 60% in saliva. *Pf.*LDH was100% in both plasma and saliva (Table 4.5).

Table 4.5 Efficiency of Detection of *P. falciparum* Antigens (HRP-2 and LDH) using ELISA-based Assay in Children and Adults.

Malaria positive by ELISA	Sensitivity	Specificity	Positive predictive value	Negative Predictive Value	n
Children					
*Pf.*HRP-2 in plasma	78%	40%	72%	48%	74
*Pf.*HRP-2 in saliva	13%	95%	86%	34%	74
*Pf.*LDH in plasma	15%	100%	100%	38%	74
*Pf.*LDH in saliva	7%	100%	100%	34%	74
Adults					
*Pf.*HRP-2 in plasma	75%	100%	100%	67%	20
*Pf.*HRP-2 in saliva	50%	60%	85%	21%	20
*Pf.*LDH in plasma	0%	100%	0%	35%	20
*Pf.*LDH in saliva	8.3%	100%	100%	35%	20

Sensitivity, specificity, positive and negative predictive values for *Pf.* Antigens (*Pf.*HRP-2 and *Pf.*LDH): ELISA results were compared to results for microscopy (gold standard)

CHAPTER FIVE

DISCUSSION

5.1 Relationship between Parasite Density, Clinical Malaria and Age Versus the Levels of Pf.HRP-2 and Pf.LDH in Saliva and Blood of Individuals with Acute Malaria Infection

Parasite density did not predict *Pf.*HRP-2 and *Pf.*LDH levels in the plasma and saliva of individuals with acute malaria infection. Previous studies reported that microscopy only measure peripheral *parasitemia* (Dondorp et al 2005). Sequestration of plasmodium parasites is a feature of clinical malaria (Chan et al., 2014). that may result in lack of estimation of the patient's total parasite biomass by the microscopist, perhaps explaining why parasite density by microscopy did not predict antigen levels in plasma and saliva of individuals with acute malaria infection in this study. Another plausible explanation may pertain to the repetitive epitopes present in *Pf.*HRP-2 and *Pf.*LDH that allows several antibodies to bind to a single protein (Kifude et al 2008). *Pf.*HRP-2 contains multiple B-cell epitopes arranged in tandem repeats of AHHAAD interspersed with AHH and AHHAA (Kifude et al 2008). This would allow binding of several antibodies to a single antigen and in turn lower the ELISA signal. This would in turn prevent true reconciliation between malaria antigen levels and parasite density by microscopy. In addition, the difference in complexity of *P. falciparum* antigens including deletion of the *Pf.*HRP-2 gene (Houzé, et al., 2009) i. may further explain why parasite density did not predict *P. falciparum* antigen levels. Furthermore, the study site was in a malaria endemic region where exposure is intense and all year round (Jenkins et al., 2015). It is possible that some of the antigen measurements were from persistent asymptomatic and submicroscopic infections that are

prevalent in malaria holoendemic areas (Sutherland et al., 2014). The contribution of clearance of circulating antigen (Vendrell et al 2020) or due to gametocytemia (Tangpukdee et al 2008) or dead parasites persisting after treatment cannot be ignored. The implication is that some of the titers of *P. falciparum* antigens measured were not from the present infection detected by microscopy. The circulating antigens may result into false positive results especially in samples obtained from children with relatively lower levels of the antigens compared to an active infection. This could explain the lack of relationship between antigen levels and clinical malaria. In contrast, a study done in children with acute and severe forms of malaria in Madang Province, Papua New Guinea (Koepli et al 2015) that measured *Pf.*HRP-2 in plasma by ELISA and compared these levels to parasite density by microscopy in a multivariate analysis showed that peripheral parasitemia predicted plasma *Pf.*HRP-2 concentration. It is possible that the involvement of patients with severe forms of disease, hence high parasitemia (Gonçalves et al., 2014) accounted for the difference between the present study and the Papua New study since the present study included only individuals with acute malaria infection.

Interestingly, age significantly predicted *Pf.*HRP-2 levels in plasma but not in saliva. Equally, age did not predict *Pf.*LDH levels in both plasma and saliva. Previous studies have showed that parasite density decreases across ages (Ototo et al., 2017). It is maximum in young children and decreases with increasing age (Koepfli et al 2015). Also, malaria antigen levels increase with increasing parasite density (Dondorp et al., 2015). It was thus anticipated that both *Pf.*HRP-2 and *Pf.*LDH levels would be higher in children than adults, however this was not observed with *Pf.*HRP-2 antigens. The thresholds for parasite density used to define a clinical case of malaria have been shown in prior studies to vary with age. It is higher in adults compared to children (Afrane et al., 2014). The observed low threshold parasite density in older patients may be due to

acquired immunity developed by virtue of repeated exposure to malaria parasites over the years(Afrane et al., 2014)). Also, the naïve nature of the immune status coupled with low blood volume and other comorbid factors such as malnitrition may make children to be more susceptible to malaria (Ilesanmi et al.,2017). These parasites release both *Pf.*HRP-2 and *Pf.*LDH into circulation and may explain why the mean levels of *Pf.*HRP-2 in plasma was higher in adults than in children in this study. These results were statistically significant. The current study has also shown that *Pf.*HRP-2 is produced in relatively larger quantities in plasma than in saliva. These lower levels of *Pf.*HRP-2 in saliva may explain the lack of variation of the antigen with age. It is also notable that there was no pattern in the variation of *Pf.*LDH levels with age. This could partly be attributed to differences in concentration (Lawrence et al., 1995) and rapid clearance of *Pf.*LDH compared to *Pf.*HRP2 (Iqbal et al., 2004). *Pf.*HRP-2 has been shown to be produced in relatively larger quantities than *Pf.*LDH (Jang et al., 2018). The antigen also persists in circulation hence still detectable even after clinical symptoms of malaria have disappeared (Houze et al 2009). Although *Pf.*HRP-2 persist after artemisinin, *Pf.*LDH levels rapidly decline after treatment hence the lack of variation of *Pf.*LDH antigen in both plasma and saliva with age. *Pf.*HRP-2 is produced in higher proportions than *Pf.*LDH and thus require a longer duration time to clear compared to *Pf.*LDH (Houze et al., 2009). Also, the production of *Pf.*LDH is related to parasite viability. The rapid disappearance of *Pf.*LDH in blood after treatment may be due to parasite death following adequate treatment (Houze et al., 2009). In addition, this study revealed a drug-related effect on the duration of *Pf.*LDH -based test positivity after treatment in both plasma and saliva

5.2 Correlation of Pf.HRP-2 and Pf.LDH Antigen Levels in Plasma and Saliva

This study investigated the link between Pf.HRP-2 and Pf.LDH levels in plasma and saliva of malaria patients to determine whether saliva might be used as a diagnostic tool instead of plasma. There was no correlation in levels of *P. falciparum* antigens in plasma and saliva of malaria infected patients. Due to low detection limit of the antigens in saliva by ELISA, saliva samples compared to plasma may have yielded false negative results (Sutcliffe et al., 2021). Reducing the number of false negatives should be the main goal of future research. Furthermore, Pf.HRP-2 and Pf.LDH are not as stable in saliva as they are in plasma. In comparison to plasma, saliva possesses proteases that may help break down the enzymes (Fung et al 2012). This may lower the levels of *P. falciparum* antigens in saliva. To lower the activity of proteases, the current study added protease inhibitors to saliva samples. Furthermore, a combination of free and antibody-bound antigen may be present in the saliva of people with confirmed P. falciparum infection. The ELISA signal would only be produced by the free fractions of Pf.LDH antigens (Nambati et al., 2018). This could further explain the results obtained. In addition, the transport of a protein into saliva depends on its molecular mass, solubility, ionization and the salivary pH. Different molecules can experience varying degrees of dilution during transfer from plasma to saliva (Fung et al., 2012)

Notably, a strong positive correlation in the levels of *Pf.*HRP-2 in blood and saliva after treatment was observed. This may be indicative of residual levels of *Pf.*HRP-2 antigens after treatment (Michael et al., 2021). indicating recent exposure to *Plasmodium (*Houze et al 2009). Since previous studies have shown that *Pf.*HRP-2 persist in patients' blood after an active infection (Fung et al 2012), the marker most likely survives in saliva as well. The implication is that *Pf.*HRP-2 levels in saliva can be used to show recent exposure to *Plasmodium* especially in

studies monitoring disease outcome. Because of this, developing saliva diagnostic technology is worthwhile. The cause of persistent malaria antigens following artemisinin are not clear. However, a possible cause might be the parasite remaining viable and asexual below the microscope detection threshold, rheumatic causes, or the parasite being affected by an antimalarial drug (Iqbal et al., 2004).

Contrary to the results above, a study carried out in Kwale county, Msambweni, to determine the correlation in the levels of *Pf.*LDH antigens in blood and saliva indicated a slight linear positive relationship (Nambati et al 2018). This study used a relatively larger sample size (175) participants unlike the current study.

5.3 Efficiency of Detection of P. falciparum Antigens using Blood-based RDT and Saliva-based ELISA Assay in Children and Adults

To determine the efficiency of detection of malaria antigens using blood-based RDTs in children and adults, Sensitivity, specificity, positive and negative predictive values for the detection of malaria antigens across ages was determined. Children reported slightly lower sensitivity (98%) compared to adults (100%), very low specificity (28%) than adults (83%). Positive predictive value was also lower in children than adults, (73%) and (88%) respectively. Negative predictive value was (92%) in children and (100%) in adults respectively. This lower sensitivity of *Plasmodium falciparum* antigens in children may be as a result of prozone effect (Maltha et al 2013). The possibility of gene deletion in isolates lacking the *Pf.*HRP-2 gene (Prosser et al., 2021) may contribute to the lower specificity of detection observed in children in this study This would further yield false negative results (Sowunmi, 2011) despite the high parasite density reported in children from previous studies (Ototo et al 2017). Reports of false negative interpretation are serious since the diagnosis of malaria may be missed. Also, an infection with

P. falciparum will be diagnosed as a non-falciparum species. Furthermore, regardless of the parasite density, age-dependent immunological status may reduce the RDT's sensitivity and specificity (Laurent et al 2010) hence the lower sensitivity and specificity of detection of malaria antigens in children compared to adults. In addition, previous studies have shown that the performance of blood-based RDTs are affected by extreme temperatures and humidity which is easily exceeded in tropical settings as in the current study (Maltha et al 2013). Only a few RDT products are able to withstand temperatures up to 40ºC, however most of the products are labelled to be stable up to 30ºC (Maltha et al 2013). These elevated temperature levels may interfere with the overall performance of blood-based RDTs. Furthermore, it was difficult to obtain sufficient information on recent past-malarial treatment especially from children who formed a majority of the samples in this study. Due to the Pf.HRP-2 antigen's persistence in circulation after the parasite dies, which is likely to have been more common in younger age groups, the majority of the reported positive results may be due to this (Mtove et al., 2011). This may further lower the efficiency of detection of malaria antigens across ages. In contrast, a study that evaluated the effectiveness of RDTs based on Pf.HRP-2 across age ranges in Southern Tanzania (Laurent et al. 2010) revealed that test sensitivity declined in older adults but increased in those under the age of 25. This could be due to the high parasite density present in children compared to adults from previous findings.

The current study hypothesized that the efficiency of detection of *Plasmodium falciparum* antigens would be enhanced by use of saliva samples. Therefore, measurements of *Pf.*HRP-2 and *Pf.*LDH antigens by ELISA were compared in the saliva of children and adults. Similar to blood-based RDTs, the efficiency of detection of malaria antigens by ELISA was still slightly lower in children compared to adult hence prompting the need for further investigations.

CHAPTER SIX

SUMMARY OF THE FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of the findings

The present study demonstrated, first, that parasite density and clinical malaria is not a predictor of *Pf*.HRP-2 and *Pf*.LDH levels in plasma and saliva of children and adults with acute malaria infection. However, age significantly predicted *Pf*.HRP-2 and not *Pf*.LDH levels in the plasma of individuals. Second, levels of *Pf*.HRP-2 and *Pf*.LDH in plasma and saliva are not correlated. Third, the efficiency of detection of malaria antigens using blood-based RDTs was lower in children than adults. Equally, the efficiency of detection of malaria antigens in saliva using ELISA assay was lower in children than in adults.

6.2 Conclusions

1. Parasite density and clinical malaria did not predict *Pf*.HRP-2 and *Pf*.LDH antigens in the plasma and saliva of individuals with acute malaria infection. This may be attributed to high number of sequestered parasite and repetitive epitopes present in *Pf*.HRP-2 and *Pf*.LDH among other factors. Age was a predictor of *Pf*.HRP-2 antigens in plasma possibly due to the high threshold parasitaemia used to predict clinical malaria in adults compared to children. However, *Pf*.LDH did not predict *P. falciparum* antigens in both plasma and saliva probably due to the lower levels of the antigens produced in circulation relative to *Pf*.HRP-2.

2. Although previous studies have shown the potential to use saliva as a non-invasive body fluid, there was no significant relationship in the levels of *Pf*.HRP-2 and *Pf*.LDH obtained in this study. This may be due to the high number of false negative results obtained in saliva and lack of stability of *Pf*.HRP-2 and *Pf*.LDH in saliva relative to plasma.

3. The efficiency of detection of malaria antigens in plasma using blood-based RDTs and saliva by ELISA was lower in children compared to adults. This may be due to a possible prozone effect.

6.3 Recommendation from current study

1. Measurement of *Pf.*HRP-2 and *Pf.*LDH in plasma and saliva may not be a good proxy measure of infection and clinical disease in populations exposed to endemic malaria. However, plasma levels of *Pf.* HRP2 can be a proxy measure for cumulative exposure in holoendemic malaria areas
2. Measurement of *Pf.*HRP-2 and *Pf.*LDH in saliva cannot substitute for the measurement in plasma hence plasma-based assays are more reliable for detection of *Plasmodium* infection in endemic areas.
3. Efficiency of detection of malaria antigens in holoendemic areas may not be improved using saliva samples prompting the need for further research.

6.4. Recommendation for future studies

1.It will be useful to compare the results for parasite density by microscopy with those achieved by more sensitive methods such as lateral flow RDTs and PCR, which measure both peripheral and sequestered parasites.

2.Further studies should be focused towards reducing the number of false negatives and developing a customized assay specific for detecting *Pf.*HRP-2 and *Pf.* LDH in saliva

3.More sensitive methods that measure circulating *Pf.*HRP-2 and *Pf.*LDH other than blood-based RDTs and saliva-based ELISA may be developed and applied to increase estimates of the total parasite biomass.

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APPENDICES

APPENDIX I: Preparation of protease inhibitors

a) Preparation of Aprotinin

50 tubes containing aprotinin stock solution and 20 tubes containing aprotinin working solution were first labelled appropriately.

Preparation of stock solution (Aprotinin lyophilized, 1mg, sigma Aldrich, Lot A1153, stored at 2-8°C)

i. About 1mg Aprotinin was placed into a bottle containing 1mL of distilled water

ii. The solution was then mixed thoroughly

iii. About 20uL of the solution was aliquoted into 1.8mL of 50 cryotubes

iv. The solution was then stored at -20°C.

Preparation of working solution

i. About 993uL of distilled water was pipetted into a 1.8mL cryotube.

ii. 6.5uL of the stock solution was obtained and added into the solution containing distilled water.

iii. The mixture was then vortexed thoroughly

iv. Using a micropipette, about 50uL of the solution was pipetted and aliquoted into 20 cryotubes of volumes 1.8mL.

v. The tubes were labelled as tube 1-20

vi. The solutions were stored at -20°C

b) Preparation of Phenylmethylsufonyl Fluoride (PMSF)

Preparation of stock solution (10mg/mL, white powder, 5g, My biosphere, CAT MBS 545833, stored at 27°C).

i. About 40mg of PMSF was weighed and placed into a 15mL plastic tube.

ii. About 4mL volume of isopropyl alcohol was pipetted and added to the powder.

iii. The solution was then vortexed thoroughly and stored at -20°C

Preparation of working solution

i. About 0.5mL of the stock solution was added into each of the eight labelled tubes

ii. The solutions were then stored at -20°C.

c) Preparation of Na_2OV_4, 400mM pH 10 in water

Reagents Required

a) Sodium hydroxide, (NaOH), Lot BCBB1911, 500g, Sigma Aldrich

b) KH_2PO_4- CAS 7778-77-0, Merck, 1kg

c) Na_2HPO_4-SO 03390500, 500g, CAS (10028-24-7) Scharlau.

Preparation of stock solution

i. About 5.678g of Na_2HPO_4 was weighed and transferred in a 100mL volumetric flask

ii. 20mL of distilled water was added and stirred using a clean spatula until all the salt was dissolved.

iii. About 5.44g o KH_2PO_4 was weighed and added to the solution in the flask

iv. The solution was stirred until all the salt dissolved

v. The solution was topped up to a 100mL mark using distilled water.

vi. The solution was poured into the labelled 100mL volumetric flask and corked using aluminum foil.

vii. The solution was stored in a cool place away from sunlight.

viii. About 2mL solution of NaOH (correcting fluid) was prepared

ix. About 8g of NaOH was weighed and placed into a 250mL glass beaker.

x. The solution was topped up to 100mL mark with distilled water

xi. The solution was mixed thoroughly

xii. KH_2PO_4 and Na_2HPO_4 were transferred into a 50mL beaker

xiii. Using a dropper, drops of solution of NaOH were added to the mixture containing KH_2PO_4 and Na_2HPO_4

xiv. While stirring gently using the pH meter, this procedure was continued until a pH of 10 was achieved.

xv. 100mL of the solution was transferred into 100mL labelled volumetric flask

xvi. The solution was corked appropriately.

Preparation of the working solution.

i. About 0.147g of Na_2OV_4 was weighed and placed in a 5mL cryotube

ii. 2mL of volume of water pH 10 was pipetted into the tube

iii. The solution was then vortexed thoroughly and stored at -20°C

APPENDIX II: An Antibody Sandwich ELISA to Detect Soluble *Pf*. HRP2 and LDH in Plasma /Saliva Supernatant.

a) Coating of Micro-titre Plates

Microtiter plates were coated with 50μL/well of diluted capture antibody in coating buffer (PBS) as follows:

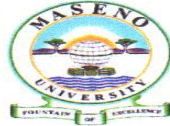
	HRP2 - CAT#532523	**1.62µL of antibody into 10mls coating buffer (for 2 plates)**
	LDH -CAT # MBS1493932	**7.14µL of antibody into 10mls coating buffer (for 2 plates)**

b) Dilution of Biotinylated detection antibody in blocking buffer

Biotinylated detection antibody was diluted in blocking buffer, (4% w/v non-fat dry milk in PBS-T buffer) as follows:

	HRP2 - CAT#6120632	**Final concentration= 125ng/ml** **(1.25µL of antibody into 10mls blocking buffer** **(for 2 plates)**
	LDH-CAT # MBS1493932	**Final concentration= 1.25µg/ml** **(60 µL of antibody into 10mls blocking buffer** **(for 2 plates)**

APPENDIX IV Maseno University Project Approval



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 18th May, 2020

TO: Ruth Awuor Omingo
PG/MSC/SC/00048/2016
Department of Zoology
School of Biological and Physical Sciences
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00816/19

RE: *Plasmodium falciparum* Antigens (Histidine Rich Protein ii, Lactose Dehydrogenase and Aldolase) Levels in Saliva as Biomarkers for Acute Malaria in Kisumu, Western Kenya. Proposal Reference Number MSU/DRPI/MUERC/816/19

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 18th day of May, 2020 for a period of one (1) year. This is subject to getting approvals from NACOSTI and other relevant authorities.

Please note that authorization to conduct this study will automatically expire on 17th May, 2021. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th April, 2021.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th April, 2021.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

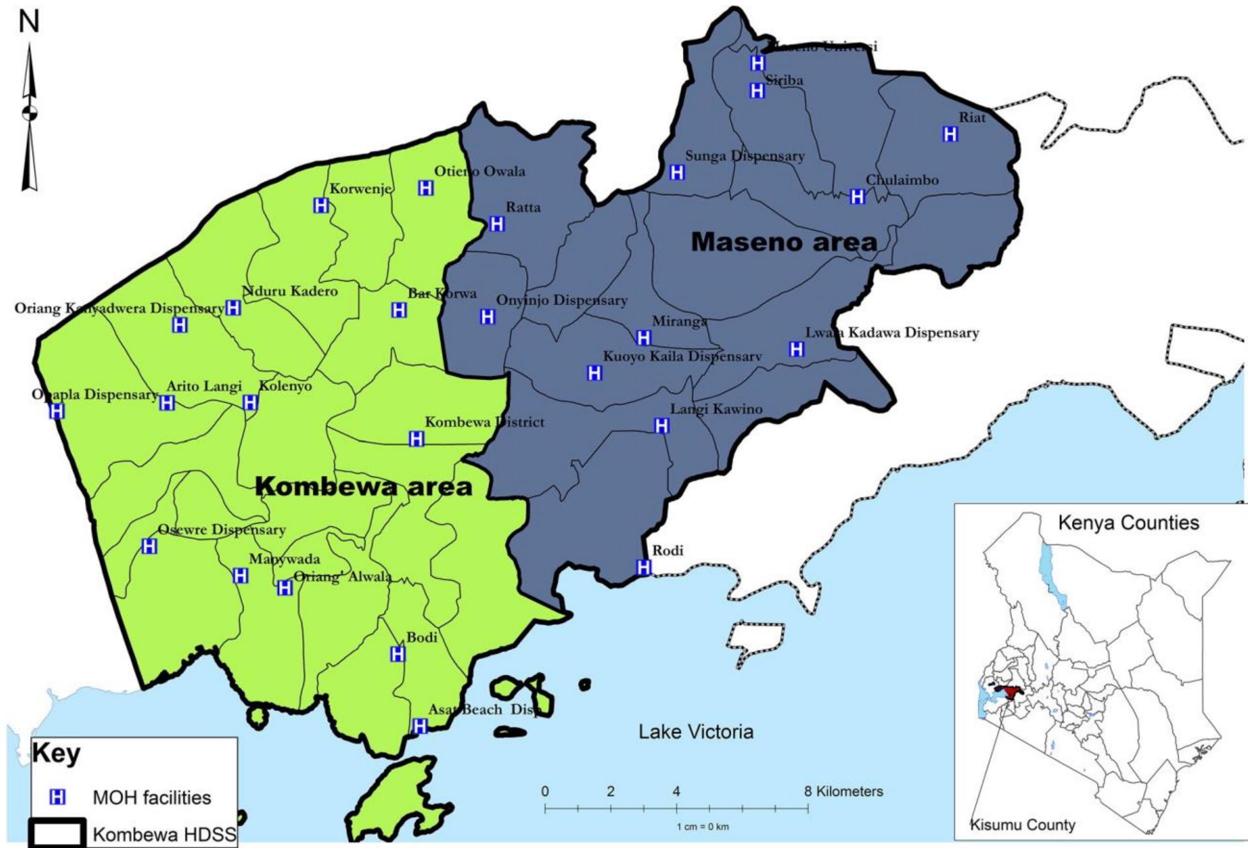
Thank you.

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

APPENDIX IV: MAP OF STUDY SITE



APPENDIX V. CONSENT FORM IN ENGLISH

Study title: *Plasmodium falciparum* antigens (Histidine Rich Protein II, Lactose dehydrogenase and Aldolase) levels in saliva as biomarkers for acute malaria.

PI Name: Ms. Ruth Awuor Omingo, Msc/Sc/00048/2016, Department of Zoology, Maseno University

Study location: Chulaimbo sub-county hospital, Western Kenya, Kisumu.

Introduction:

Hello. My name is Ruth Omingo. I am working on determining *Plasmodium falciparum* antigen (HRPII, Aldolase and Lactose dehydrogenase) levels in saliva as biomarkers for acute malaria diagnosis

Purpose of research project:

We are carrying out the research survey to assess the performance and efficiency of detection of *P. falciparum* antigens (*Pf*.HRP2, *Pf*.LDH and aldolase) levels in saliva and blood as biomarkers in the diagnosis of malaria infection using blood-based RDT and saliva-based ELISA assay across different ages.

Voluntary participation:

You/your child participation in the survey is voluntary. You/your child may withdraw or stop your participation in the study at any given time for any reason. There is no consequence for stopping to participate in the survey.

Why you are being asked to participate:

You/your child are being asked to participate because you/your child are suspected to be positive for malaria. With you/your child consent, your blood and saliva samples will provide us with information on the presence of malaria parasite in you/your child saliva and blood. We will ask you/your child to provide a blood sample when you are sick with malaria. We will also ask you/your child for a second sample eight weeks after you recover. If we are drawing blood from you and your child, we will take approximately a quarter tea spoon (0.5-1ml). We will collect the blood sample by pricking you or your child's finger with a lancet and squeezing the finger to draw blood. For saliva, we will give you/your child a tube in which we request you to collect 1-2 teaspoonfuls of saliva (5-10) ml. The blood and saliva samples will be transported to Maseno University for storage and analysis. Additional analysis will be done at KEMRI-KISIAN. If you/your child are found to be sick with malaria, we will refer you to Chulaimbo Health center for treatment, however we will not cover the cost for treatment.

Risks:

You/your child may experience some little discomfort and pain during a finger prick. However, this procedure will be carried out by well qualified personnel to minimize pain and any form of injury that may be experienced during the procedure. If you are pregnant, there will be no additional risk to the fetus. We will also do our best to keep all information that you/your child provide us in this survey confidential.

Benefits:

There are no direct benefits, monetary or otherwise, associated with you/your child's participation in the study. However, it is anticipated that participants will gain future benefits should there be interventions or policy changes as a result of the research project.

Confidentiality:

All information you/your child provide us during the survey will be stored confidentially. In order to make sure that you/your child's privacy is safeguarded, we will not reveal you/your child personal details such as you/your child's name to any individual who is not part of this study. Only study personnel and institutional review board will be allowed access to the information collected in this study.

Long term Storage of samples

We are also requesting you/your child's permission so that we can store any samples of your or your child's saliva or blood that may remain after the study. The samples will be stored at Zoology department of Maseno University. The samples will be used for future studies to analyze the complete protein composition of saliva and how it affects malaria diagnosis. Any of these future studies will l be done upon seeking approval from Maseno university ethical review committee.

As a participant, you have the right to withdraw you/your child agreement to use samples for future research, at any time for any reason. If you/your child withdraw, the sample will be destroyed.

If you agree tick YES below, if you don't agree tick NO below.

Do you have any questions?

Who do you call if you have any questions or problems?

You can contact **Ms. Ruth Omingo at 0725-946-180: Dr. Cyrus Ayieko on 0720852927**

You can also call the **Secretary of the Maseno University Ethics Review Committee,** Private bag,

Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:

Muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com

What does your written consent mean?

Your written consent means:

1. You have been informed about this study's purpose, procedures, possible benefits and risks.
2. You have been given the chance to ask questions about this study
3. You have voluntarily agreed that you/your child should participate in this study

Individual consent to participate in the survey

Name of participant____

Date________________

Signature of participant/child signature__________________________

Date________________

Signature of guardian or parent ____________________________________

Date________________

Relationship to the participant______________________________________

Date________________

Signature of person Obtaining Consent____________________________

Date________________

Signature of the principle investigator_____________________________

Date________________

Give a copy of the signed consent form to the participant and keep one copy in study records.

End of consenting process

APPENDIX VI: CONSENT FORM IN DHOLUO
BARUP YIE BEDO E NONRO

THUON WACH: NONRO MAR KIWANGO MAG ANTIGENS (HISTIDINE RICH PROTEIN 2,LACTATE DEHYDROGENASE KOD ALDOLASE)MAR MALARIA EI ALAU KAKA RANYISI MAR TUO MAR MALARIA

NYING JATIM NONRO: MADAM RUTH AWUOR OMINGO, MSC/SC/00048/2016, DEPARTMENT MAR ZOOLOGY , MBALARIANY MA MASENO

LOKESEN MA ITIMOE NONRO: OSIPTAL MA CHULAIMBO, WESTERN KENYA, KISUMU

CHAKRUOK

Nyinga en Ruth Omingo, atimo nonromar ng'eyo kiwango mag antigens mag tuo malaria ei olau kaka ranyisi mar malaria

EN AN'GO MA OMIYO WATIMO NONRO NI?

Watimo nonro mondo wang'e kaka antigens mag malaria tiyo e del kendo yot mar yudo gi e I olau kod remo ka watiyo gi kits mag remo mag malaria gi ELISA e kind mag higni mopogore opogore

YIE E NONRO MAONGE ACHUNA

Yie mari kata mar nyathini e nonro ni onge gi achuna moramora,inyalo dagi yie kaw thuolo mari kata mar nyathini e nonroni. Onge rach mora mora ma nitiere ka itamori kata nyathini yie bedo e nonroni

EN ANG'O MA OMIYO IKWAYI MONDO IKAW THUOLO E NONRONI?

Mokwongo wachich ni in kata nyathini nyalo bedo gi malaria.Gi yieni kata mar nyathini,wabiro kwayo mondo wakaw remo gi olau matin mari kata mar nyathini mabiro nyisowa ranyisi mag malaria.Wabiro kwayi kata nyathini kendo remo gi alau matin bang jumbe aboro ka in kata nyathini osechango.Ka wakau remo mari kata mar nyathini, wabiro dwaro mana kijiko matin mar chai mar remo.

Bende wabiro miyi kata nyathini chupa matin kendo kwayi kata nyathini mondo ong'udhe olau madirom kijiko ariyo.

Remo kod olau mawasekaw wabiro tero e mbalariany ma Maseno mondo wakan kendo watimne nonro.Nonro moko matut wabiro timo KEMRI ma Kisian.

Ka wayudo ka in gi malaria to wabiro kwani mondo ichop e osiptal machiegni mondo iyud thieth to kata kamano ,chudo mari kata mar nyathini mar thieth to ibiro timo kendi.

RACH MAR NONRONI

In kata nyathini biro winjo rem matin sama wagolo remo to kata kamano wabiro ibiro time jalony mondo rem kata chandruok kik bedie ahinya.To ka ipek to onge rach kata chandruok ma nyathima iting'o biro yudo,wach moramora ma ichiwonwa wabiro rito makare kendo gi siri mang'eny.

BER MAR NONRONI

Ber ma oyangore mar pesa kata yie mari kata mar nyathini to onge,to kata kamano wa gi yie mathoth ni e kinde mabiro ni joma oyie kaw kindegi e nonroni biro yudo konyruok maduong ka yorekod chike mag pimo malaria oloki nikech nonroni temo tiyo gi olau kaka yor manyo malaria.

SIRI MAR NONRONI

Wach moramora obed ma in kata nyathini ondiko kata owacho wabiro rito makare.Siri mari kata mar nyathini chakre nyinge kata kuma oayebende wabiro rito makare.Ok wanahul ne ng'ato moramora ma ok achiel kuom joma timo nonroni.Joma nigi lony kata kamiti ma nono nonroni e ma oyiene neon weche mag nonroni,

KENO MAR REMO GI OLAU MAWAYUDO KUOM KINDE MATHOTH

Wakwayo mondo wakan remo kata olau modong e nonroni,wabiro kanogi e mbalariany ma Maseno e apisi mare mar timo nonro gi le.Wabiro tiyo kodgi kuom nonro mabiro nyime mando wamed nono antigens te mayudore e alau kendo kaka gi konyo e yor ng'eyotuo mar malaria.

Nonro moramora mabiro nyime ibiro timo mana mana ka wakaw thuolo kuom jolony manono nonro mitimo e mbalariany

Kaka ng'at ma okaw thuolone e nonroni,oyieni kata nyathini mondo idagi tiyo gi rembi kod alapi kata mar nyathini ne nonro mabiro nyime.Ka in kata nyathini odagi to wabiro kethogi kendo weyo tiyo kodgi kuom nonro mabiro

Ka iyie to gweth tik kama ondik ni AYIE, to ka idagi to gweth tikkama ondik ni ADAGI

AYIE……………………………………
ADAGI…………………………………….

Be in gi penjo moramora?

Ng'at mane ma inyalo yuane tol ka in gi penjo kata chandruok moramora?

Inyalo gochona….. Ms. RUTH OMINGO: 0725946180

CYRUS AYIEKO DAKTARI: 0720852927

Bende inyalo yuayo tol ne jandiko mar apisi mar jolonye weche mag nonro ma mbalariany ma Maseno

057-51622, 0722203411, 0721543976, 0733230878.

Kata igwelwa e mbui: Muerc.secretariate@maseno.ac.ke; muerc.secretariate@gmail.com

KA IYIE BEDO E NONRONI TO NYISO NANG'O?

1.Osenyisi kuom nonroni ,kaka ibiro timo,ber mage kaachiel gi rach mage

2.Osemiyi thuolo mar penjo penjo moramora

3.iseyie maonge achuna mondo ibed achiel e nonroni

Nying ng'at ma oyie…………………………………………..…………….
TARIK……………………………………

Sei mari kata mar nyathini……………………………………..……………
TARIK……………………………………

Tudruok mari gi ng'at ma oyie…………………………………………..….
TARIK……………………………………

Sei mar ng'at makaw baruwani………………………………………………
TARIK………………………………….

Sei mar jalony maduong e nonroni…………………………………………
TARIK………………………………..

MI NG'ATMA MA OKAW THUOLONE E NONRONI BARUA ACHIEL KENDO KAN ACHIEL E KAR KENO MAR NONRONI

GIKO MAR BARUA MAR YIE BEDO E NONRONI.

APPENDIX VII: CONSENT FORM IN KISWAHILI

FOMU YA KUBALI

MADA YA UTAFITI

Viwango vya antijeni za vimelea vya malaria (*Plasmodium falciparum* Histidine Rich Protein 2, Lactose dehydrogenase and aldolase) kutumia mate kama chombo cha utambuzi.

JINA LA MCHUNGUZAJI MKUU: Madam Ruth Awuor Omingo, Msc/Sc/00048/2016, Depantmetia ya Zoology, Chuo Kikuu Cha Maseno.

ENEO LA UTAFITI: Hospitali ya kaunti ndogo Chulaimbo, Magharibi Kenya, Kisumu.

UTANGULIZI

Hujambo, jina langu ni Ruth Omingo.Kiini cha utafiti wangu ni kuamua kiwango cha vimelea vya mbu vinavyo sababisha ugonjwa wa malaria kwa kutumia mate kama chombo cha utambuzi.

KIINI CHA UTAFITI

Tunafanya utafiti huu ndio tuweze kujua utendaji na ufanisi wa kugundua viwango vya antijeni za vimelea vya malaria (PfHRP2,LDH na Adolase) kutumia damu na mate kama vyombo vya utambuzi wa malaria kutumia vipengele au misingi ya damu ya RDT na uchunguzi wa mate kutumia msingi wa ELISA kwa watu wa umri tofautitofauti.

USHIRIKI WA HIARI

Kujihusisha kwako ama mototo wako katika utafiti huu ni wa hiari. wewe ama mototo wako mnaweza jkutoka au kuacha kushiriki utafiti huu wakati wowote kwa sababu yoyote. Hakuna madhara yoyote kwa kutoshiriki katika utafiti huu.

MBONA WAULIZWA KUSHIRIKI?

Wewe au mwanao mnaombwa kushiriki katika utafiti huu kwa sababu mmehutumiwa kuwa chanya kwa ugonjwa wa malaria. Kuwepo kwako au mwanao kutatuwezesha kuchukua sampuli ya damu na mate ambazo zitatusaidia kupata ujumbe huhusu uwepo wa vimelea vya malaria kwenye damu ama mate. Tutaomba wewe au mwanao sampuli za damu na mate mnapougua malaria. Pia tuthitaji sampuli ya pili ya mate na damu wiki nane baada ya kupona. Tutatoa damu takriban robo kijiko cha chai (0.5-1ml) kwako au kwa mwanao. Tutakusanya sampuli ya damu kwa kudunga kidole chako au mwanao kwa kutumia lancet (kasindano) na kufinya kidole ili kutoa damu. Pia tutakupa wewe au mwanao mrija wa kuweka/kutema sampuli ya mate takriban kijiko 1-2(5-10ml) ndogo cha chai.

Sampuli ya mate na damu zitasafirishwa mpaka chuuo kikuu cha maseno kwa huhifadhi na kufanyiwa uchanganuzi. Uchanganuzi zaidi utafanya katika chuo cha utafiti wa sayansi cha

KEMRI-KISIAN. Iwapo mtapatikana na malaria tutawatuma katika hospitali ya Chulaimbo kwa matibabu zaidi, ila hatagharamia malipo ya matibabu mwenyewe.

HATARI

Katika harakati ya kutoa damu,wewe au mwanao mnaweza huhisi uchungu kiasi, lakini mtindo huu utafanyawa na mtaalamu katika utoaji wa damu. Iwapo umjamzito, hakutakuwepo na hatari yoyote kwa mototo. Pia tutajitahidi tuwezavyo kuweka ujumbe wako au wa mwanao kisiri.

FAIDA

Hakuna manufaa ya kifedha utakayopata kwa kushiriki katika utafiti huu,ilhali tunahuhakika kuwa washiriki watapata manufaa siku sijazo iwapo utafiti huu utakubalika ama kuwe na mabadiliko ya sera za utafiti huu.

USIRI

Ujumbe wote utakaotupa ama wa mwanao utawekwa kisiri. Ili kuhakikisha kwamba faragha yako ama ya mwanao imelindwa hatutadhihirisha ujumbe wenuwa kibinafsi kama jina la motto kwa mtu yeyote asiyehusika katika utafiti huu. Kipekee, ni wale wanaoshiriki katika utafiti huu na kamitii ya ukaguzi ndiyo utaruhusiwa kupata ujumbe huu.

UHIFADHI WA SAMPULI KWA MDA MREFU

Tunaomba idhini yako au mwanao kuhifadhi sampuli ya damu na mate zitakazobaki baada ya shughuli za utafiti. Sampuli zitahifadhiwa katika idara ya Zoologia ya chuo kikuu cha maseno. Sampuli hizi zitatumika kwa utafiti wa siku zijazo kwa uchananuzi wa ukamilifu wa protini katika mate vile zinathiri utambuzi wa malaria.utafiti wowote utanywa baada ya kutafuta idhini kutoka kwa kamitii ya madili ya tathmini ya chuo kikuu cha maseno.

Kama mshiriki, una na haki ya kutoa ridhaa yako au ya mwanao ya kutotumia sampuli katika utafiti wa baadaye wakati wowote kwa sababu yoyote. Ukitoa ridhaa yako ama ya mwanawe sampuli zitaharibiwa.

Kama umekubali weka alama ya pata hapo chini kwa kisanduku cha **NDIO**. Kama hujakubali weka alama ya pata kwa kisanduku cha **HAPANA.**

UNA SWALI LOLOTE?

UTAMPIGIA NANI SIMU UKIWA NA SWALI AU SHIDA YOYOTE?

Unaweza pigia :

Ruth Omingo : 0725-946-186

Daktari Cyrus Ayieko: 0720852927

Secretary E.R.C Maseno private bag: 057-51622, 0722203411, 0721543976, 0733230878

EMAIL: muerc.secretariate@maseno.ac.ke, muerc.secretariate@gmail.com

FOMU YA KUBALI ANDIKWA INAMANISHA NINI?

Inamanisha:

1. Umejulishwa kuhusu nia ya utafiti huu , mtindo na manufaa wezekano.
2. Umepewa nafasi ya kuuliza maswali kuhusu utafiti huu.
3. Kwa hiari umekubali kuwa wewe au mwanao anapaswa kushiriki katika utafiti huu.

Jina la mshiriki………………………………………

Tarehe …………………………………………..

Sahihi ya mshiriki/sahihi ya mtoto……………………………
Tarehe……………………………………………………..

Sahihi ya mzazi/mlezi…………………………………………

Tarehe ………………………………………………………..

Uhusiano kwa mshiriki…………………………………………

Sahihi ya anayepata kubali………………………………… ….
Tarehe………………………………………………………..

Sahihi ya mchunguzaji mkuu (PI)……………………………

Tarehe………………………………………..

Mpe mshiriki nakala moja ya fomu ya kubali iliyotiwa sahihi, weka nakala ingine kwa rekodi za utafiti

APPENDIX VIII: Assent form

Project Title: *Plasmodium falciparum* antigens (Histidine Rich Protein II, Lactose dehydrogenase and Aldolase) levels in saliva as biomarkers for acute malaria. **Investigator**: Ms. Ruth Awuor Omingo, Msc/Sc/00048/2016, School of Biological and Physical Sciences , Department of Zoology, Maseno University.

We are doing a research study about determining the levels of malaria parasite antigens in saliva and blood and hoping to use this information to diagnose malaria infection using blood-based RDT and saliva-based ELISA assay across different ages.

Research purpose: A research study is a way to learn more about people. If you decide that you want to be part of this study, you will be asked to donate your blood and saliva samples which will be used in the above study.

Risks: There are some things about this study you should know: During the process of obtaining especially your blood sample, you may experience a little discomfort. However, the procedure will be carried out by qualified phlebotomist so as to reduce the chances of injuries that may be experienced throughout the study.

Benefits: No one taking part in this study will benefit. A benefit means that something good happens to you. However, it is anticipated that you who is participating in the study will gain future benefits should there be interventions or policy changes as a result of the research project.

When we are finished with this study, we will write a report about what was learned. This report will not include your name or that you were in the study.

You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that's okay too. Your parents know about the study too.

If you decide you want to be in this study, please sign your name.

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

____________________________________ ________________

(Sign your name here) (Date)

APPENDIX IX: Clinic form

Age of participant

___

Place of residence

___

Phone number of participants

___

Participant's identification number (study number)

___

Symptoms for malaria

___

___

Malaria positive? (YES/NO)

___

Last episode of malaria

___

Any other symptoms

___

___

Weight of patient

___

HB levels of patient

___

Blood pressure levels of patient

___

Any other information (AOB)

___