Asymptomatic *Plasmodium* infection in selected Low Transmission Setting of Dedo District, South-west Ethiopia

A Thesis Submitted to School of Medical Laboratory Sciences, Institute of Health, Jimma University, In Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Parasitology

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JIMMA UNIVERSITY INSTITUTE OF HEALTH SCHOOL OF MEDICAL LABORATORY SCIENCES

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Abstract

Background*: Regardless of decades of sustained control efforts, malaria remains a major cause of morbidity, mortality and socio-economic problems in Ethiopia. Even though it shows a remarkable decline in the last decade and given that elimination efforts are underway, asymptomatic infections may pose major challenges to the aimed elimination program of the country by 2030.*

Objective: *The aim of this study was to determine prevalence of asymptomatic Plasmodium infection and assess trends of malaria in selected low transmission setting of Dedo District, Ethiopia.*

Methods: *Repeated, community based cross-sectional surveys were conducted among 150 households (743 study participants) in May and October 2018 in Waro Kolobo kebele of Dedo district, Ethiopia. Pre-tested semi-structured questionnaire was used to collect data on socio-demographic characteristics of the study participants and the associated risk factors. Finger prick blood samples were taken from all study subjects to detect malaria parasite by microscopy and rapid diagnostic tests (RDT). Additionally, a retrospective study was conducted in offele health center to assess a five-year malaria trend (2014-2018) in the study area. Data were entered to Epi-data and Statistical analysis was performed using SPSS version 20. Descriptive statistics were utilized to summarize demographic profile of the study participants. Bivariate and multivariable logistic regression analysis were employed to determine the association between independent variables and the outcome/dependent variable. P-value ≤ 0.05 were considered as statistically significant.*

Results: *In the two cross-sectional surveys, a total of 743 study participants were included. In the first survey, which included 370 study participants, none of the blood samples collected and analyzed by microscopy and RDT were positive for Plasmodium infection. In the second cross-sectional survey, (6/373) of the blood samples were positive using microscopy but were negative by the RDT. Almost all the households included in the surveys had at least one LLIN. However, only 62.4 and 69.4% of the study participants used LLIN the previous night before the survey in the first and the second survey respectively. Educational status AOR (2.4, 95% CI: 1.297-4.617; p=0.006), age group (AOR 2.3, 95% CI: 1.307-4.237; p=0.004) and LLIN utilization (AOR 3.5, 95% CI: 1.964-6.266; p<0.001) were significantly associated with history of malaria in the preceding one year.*

Conclusion: *The prevalence of asymptomatic malaria infection was 6/373 in the study area. Although the LLINs coverage is high, about a third of the residents did not use LLIN the previous night before the survey. Long lasting insecticide nets utilization assessment is required to further suppress the transmission of malaria.*

Key words: ***Asymptomatic plasmodium infection, prevalence, low transmission setting,***

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List of Acronyms

ITN	Insecticide Treated Net
IRS	Indoor Residual Spraying
LLIN	Long-Lasting Insecticidal Net
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
PvMSP	*Plasmodium vivax* merozoite surface protein
RBC	Red blood cell
RDT	Rapid diagnostic test
WHO	World Health Organization

Chapter One: Introduction

1.1 Background

Malaria is one of the most serious and widespread protozoan infections of humans. Human malaria is mainly caused by four species of parasites in the genus *Plasmodiam, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae.* With limited geographical distribution and of zoonotic importance, *Plasmodium knowlesi* also infects human (1, 2). Malaria transmission in Ethiopia is seasonal, depending mostly on altitude and rainfall. The two main seasons for transmission of malaria in Ethiopia are September to November, sometimes extended to December after heavy summer rains, and March to May, after the light rains(3).

Malaria transmission in Ethiopia is spatio-temporally dynamic, with unstable and seasonal transmission occurring in most parts of the country where transmission occurs. Perennial transmission occurs in the western lowlands of the country, where whether conditions are suitable for malaria transmission throughout the year(4). The transmission is mainly related to environmental and climatic factors including altitude and rainfall. Both *P. falciparum* and *P. vivax* are co-endemic in Ethiopia. In high transmission areas, continuous exposure to *Plasmodium* parasites leads to partial immunity and consequently, creates asymptomatic carriers (5).

Asymptomatic malaria can be defined as a case where an individual harbor the parasite and is usually capable of transmitting the disease, but without exhibiting symptoms. Asymptomatic malaria can also be seen to a lesser extent under unstable transmission conditions. Unstable malaria is typically the result of seasonal influences. There is a period when mosquito populations are at a minimum and asymptomatic infections likely become a refuge for the parasite population and the source of new infections when mosquito populations expand(5). The prevalence of asymptomatic parasitaemia depends upon endemicity of malaria in the area, period of stay in the endemic area, age, development of partial immunity by the previous repeated exposures to malaria, gender, use of bed nets, and the genetic background (6).

Asymptomatic malaria is of epidemiological interest, as it can be used as an index for evaluating the utilization and implementation of malaria vector control programs as well as monitoring their continued use (6). Asymptomatic *Plasmodium* infection is not often detectable by routine laboratory techniques. WHO recommends light microscopy as the ‘gold standard’ for symptomatic malaria, but its performance for detecting asymptomatic infections, especially under low endemic settings, is generally poor (7).

The most widely used criteria for diagnosis of asymptomatic malaria are presence of parasites in peripheral thick blood smears, an axillary temperature <37.5°C, and an absence of malaria-related symptoms(8). Asymptomatic malaria cases act as reservoirs of parasites as they may be gametocyte carriers, contributing to the persistence of malaria transmission(9).

Hence, determining the magnitude of asymptomatic infections in a particular area is of significant epidemiological importance. Sometimes the asymptomatic infections may persist long allowing continued exposure to the local mosquito vector and possibly sustaining malaria transmission in different seasons. Malaria elimination efforts are hindered by the prevalence of asymptomatic infections, which frequently go undetected and untreated(10). It is well known that malaria epidemiology varies between country and region because the dominant vector species, the characteristics of human populations and factors that influence transmission such as rainfall, temperature, housing conditions and population movement differ. Therefore, the challenges to malaria elimination in different settings will vary. Each area needs to investigate the malaria epidemiology and carefully tailor its diagnosis strategy to the local context.

For malaria elimination settings it is critical to detect all infections, including those with low and sub-microscopic parasite densities in asymptomatic carriers as they represent a parasite reservoir in the community capable of effectively transmitting infections to mosquitoes and seeding transmission foci(11).

Malaria diagnosis is traditionally achieved by microscopic examination of blood smears. Microscopy is able to detect parasite species and determine parasite densities. However, the quality of microscopy can vary significantly because its accuracy largely relies on the experience and training of the microscopists to make and stain a blood slide correctly and read it accurately(12).

Malaria rapid diagnostic tests (RDTs) have been developed and tested over the past 2 decades as an alternative to microscopy, particularly for areas where quality microscopy is absent or hard to maintain. RDTs are lateral flow devices that detect parasite proteins using antibodies. The tests are easy to perform and provide rapid results in 15 to 20 minutes without the need for electricity, expensive equipment or extensive training(13).

1.2 Statement of the problem

Globally, an estimated 3.3 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk to acquire the disease. The burden is heaviest in the World Health Organization (WHO) African Region, where an estimated 93% of all malaria deaths occur, and in children aged under 5 years, which account for 61% of all deaths(14).

In 2017, an estimated 219 million cases of malaria occurred worldwide and there were an estimated 435 000 deaths from malaria globally, compared with 451 000 estimated deaths in 2016, and 607 000 in 2010 (14). Because of the integrated actions to combat malaria in the last decade and half, almost half of the world's nations are now malaria free (14). Malaria remains an important cause of morbidity and mortality in many parts of the world. Apart from its health impacts, it has also adverse socio-economic consequences(5, 15).

According to the 2016 World Health Statistics report, 27% of the population lives in high transmission areas while 41% lives in low transmission localities. Ethiopia is among the top five malaria riddled countries in Africa. Despite the long history of malaria eradication and control since the 1950s, this disease is still a major challenge in the country (16)Malaria is a major public health problem in Ethiopia and it is estimated that over 68% of the landmass of the country is favorable for malaria transmission. About 60% of the population, estimated 60 million people live in these malaria risk areas(16).

Asymptomatic infections are often not addressed in the malaria control programs, which is usually based on the passive detection of cases in health facilities and treating them. Active case detection, which captures the asymptomatic cases, is expensive and so far, not in routine practice by the health facilities in Ethiopia. The passive case detection has its own limitations as it captures only those seeking treatment in the health facilities. However, substantial proportion of individuals in malaria endemic areas is asymptomatic (11, 17).

However, asymptomatic infections may pose problems in achieving the elimination efforts in areas of low transmission setting. Although the precise role of microscopy undetected asymptomatic malaria carriers acting as reservoirs of infection to the vector mosquitoes remains to be understood, it is of immense interest to the scientific community. A recent report indicates the possibility that asymptomatic malaria carriers can infect the vector mosquitoes (18).

Apart from their potential role as reservoirs of infection, asymptomatic malaria carriers may harbor drug resistant strains. Some studies have documented drug resistance among asymptomatic individuals(17). Drug resistance in asymptomatic individuals further complicates the elimination efforts as they may spread the drug resistant genes like, Pfcrt 76T mutation was 92% in isolates from asymptomatic patients. The Pfdhfr (codons A16V, N51I, C59R, S108N and I164L) and Pfdhps (S436A, A437G, K540E, A581G and A613S) mutations are associated with resistance to sulphoxide and pyrimethamine, respectively(19). Therefore, study of magnitude of asymptomatic infections in a particular area is crucial.

There are no written documents on asymptomatic malaria infection in the study area. Therefore, the aim of this study was to determine the prevalence of asymptomatic malaria infection and assess associated factors in Dedo district, south-west Ethiopia**.**

Chapter Two: Literature Review

Malaria is a significant public health problem globally with countries in sub-Saharan Africa harboring majority of the burden. In Ethiopia, the disease is declining over the last decade and in some area's elimination efforts are to be undertaken(14). Malaria transmission in Ethiopia is highly unstable, with 'epidemic years' occurring every 5 to 8 years. In areas of unstable transmission, the transmission occurs bi-annually. The major transmission is from September to December following the main rains from June to August. Minor transmission occurs from April to May following a short rainy season from February to March coinciding with major harvesting season with huge consequences on the economy of the country. The transmission is largely determined by climate and altitude(3)

Analysis of malaria data from healthcare system is essentially important to assess achievement or failure of malaria control in a country(20). In Ethiopia, some retrospective studies have been carried out to assess changes in malaria indices. Indeed, studying malaria trends is considered to be one of the most important aspects of employing effective control strategies in malaria-prone settings. In Ethiopia, primary health centers are the basic elements of the healthcare system which play a vital role in minimizing the malaria burden among the rural poor residing in remote areas of the country(21).

Marked spatio-temporal variation in the level of transmission and species of *Plasmodium* are the main features of malaria transmission in Ethiopia (22). *P. falciparum* is predominant in the western low land areas of the country where perennial transmission occurs. As a result of the short peak transmission and the relatively long duration of low transmission during the dry season, people are highly vulnerable to malaria due to lack of acquired immunity that comes with frequent exposure to malaria infections, resulting in the occurrence of frequent epidemics (22). Nationally, *P. vivax* and *P. falciparum* comprise about 40% and 60% of malaria infections, respectively (23).

The production of gametocytes within an infected human host is essential for transmitting the parasite to the mosquito. Understanding the variables that are involved in gametocyte production within the human host, such as the host immune response to parasites, anti-malarial drug treatment, and parasite genetic diversity, and correlating these with the presence or absence of symptoms, is layered with(24).

Complexity of malaria transmission depends on the presence of mature gametocytes in the peripheral blood. The production of gametocytes from their asexual progenitors differs between *Plasmodium* species. In *Plasmodium vivax* gametocytes, generation begins early during infection, with gametocytes appearing in the bloodstream 2–3 days after the first asexual parasites and typically disappearing within 3 days after asexual infections are cleared. Therefore, asymptomatic infections with low asexual levels may also have low gametocyte densities. Data from epidemiological studies confirm that most asymptomatic infections with patent or sub-patent asexual stage parasite levels have sub-patent gametocytemia(25).

A finding from south-east Bangladesh shows that malaria prevalence across all sub districts in the monsoon season was 30.7% and 14.2% by PCR and microscopy, respectively (26). Asymptomatic malaria infections may also tend to vary depending on some socio-demographic characteristics. For example, a study from eastern Myanmar indicates a significantly higher prevalence of asymptomatic *Plasmodium* infections in males than females (27). On the other hand, in a study conducted in Nigeria the prevalence of asymptomatic infections was not associated with gender, with no significant difference between the male and female. However, the study showed that incidence of asymptomatic malaria was highest with 76.1% in the children aged 6–10 years (6). Some countries approaching elimination have reported very few or no cases of asymptomatic infections. For example, in a study conducted in Iran in 2012 asymptomatic carriers were not detected with a substantial decline in malaria cases and low sero-positive of anti- PvMSP-119 and PfMSP-119 antibodies. This appears to be due to the malaria elimination activities carried out in the area(5) .

The early identification and treatment of asymptomatic infections might accelerate elimination efforts(28). The prevalence of asymptomatic malaria infections is heavily dependent on the methods used to detect the cases. As asymptomatic malaria infections are often submicroscopic, the usual blood film microscopy often misses the cases, underestimating the burden. In a recent study from north west Ethiopia, for example, while the prevalence was 0.7% using microscopy, qPCR detected prevalence of 12.7%, indicating that a remarkable proportion of the cases are missed by the routine blood smear microscopy (29).

Control efforts depend critically on the availability of effective diagnostic tools, particularly for the identification of asymptomatic infections, which play a key role in disease persistence and may account for most instances of transmission but often escape detection by the available screening methods(10).

Malaria rapid diagnostic tests (RDTs) are instrument-free tests that provide results within 20 min and can be used by community health workers. RDTs detect antigens produced by the *Plasmodium* parasite such as *Plasmodium falciparum* histidine-rich protein-2 (PfHPR2), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase(30).

Light microscopy is still the primary method of malaria diagnosis in endemic settings, Giemsa-stained thick blood film analysis is cheap and enables scoring of parasite density, to identify the different *Plasmodium* species and to differentiate sexual (gametocytes) from asexual stages(30).

While microscopy is still considered the gold standard, RDTs are growing in popularity as they allow for rapid and inexpensive diagnosis. Microscopy has been the method of choice in determining the prevalence of malaria in epidemiologic surveys, allowing quantification and differentiation of *Plasmodium* species at low cost. More recently, rapid diagnostic tests (RDTs) were introduced as screening tools in field based surveys, as they provide readily available results allowing for treatment in situ (31).

Accurate identification of *Plasmodium* infections in community surveys is essential to successful malaria control. Microscopy and rapid diagnostic tests (RDTs) are the main techniques used to diagnose malaria in field-based surveys. Microscopic examination of Giemsa-stained blood smears has subsequently become the gold standard of malaria diagnosis and the use of antigen detecting rapid diagnostic tests (RDTs) is a vital part of this strategy, especially where good-quality microscopy cannot be maintained(32).

Microscopy and RDT (detecting both HRP2 and pan-*Plasmodium* lactate dehydrogenase in (SD-Bioline) that utilizes 5 µL entire blood for the identification of *P. falciparum* specific histidine rich protein-2 (*P. falciparum*-HRP2), and pan-specific pLDH for all *Plasmodium* species can detect significant number of asymptomatic infections even though they are less sensitive compared to molecular techniques (11).

In recent years, the use of more sensitive diagnostic techniques has demonstrated a significant number of malaria infections at densities beneath the limit of detection of conventional microscopy and rapid diagnostic tests (RDT)(25). These low-density infections are almost always asymptomatic, found in all endemic settings, including those nearing elimination, and in all ages of the population. They typically account for a high proportion of all infections and since they have also been shown to be infectious to mosquitoes, low-density infections are thought to be important contributors to maintaining malaria transmission(28).

2.1 Significance of the Study

Asymptomatic *Plasmodium* infection is a challenge to the Ethiopian national malaria control program aiming to eliminate malaria by 2030. Determining the burden of asymptomatic *Plasmodium* infections is therefore essential. The status of asymptomatic malaria in Dedo district, was not well assessed regardless of the history of the area which has been known for its high malaria burden. This study determined the prevalence of asymptomatic malaria among residents in the area, and also trend of malaria in the study area was assessed. This could help in reducing the local parasite reservoir.

Chapter Three: Objectives

3.1 General Objective

To determine prevalence of asymptomatic *Plasmodium* infection & assess trend of malaria in selected low transmission setting of Dedo district, South-West Ethiopia

3.2 Specific objectives

- To determine prevalence of asymptomatic *Plasmodium* infection in Waro Kolobo kebele
- To assess trend of malaria in Waro Kolobo kebele
- To assess utilization of long-lasting insecticide nets among the study participants
- To assess factors associated with asymptomatic *Plasmodium* infections in the study area

Chapter Four: Methods

4.1 Study setting & period

This study was conducted in May and October 2018 in selected village of Dedo district, Jimma Zone, south-western Ethiopia. The district is located 373 Kms from Addis Ababa. It is bordered in the south by Gojeb River which separates it from the Southern Nations, Nationalities and Peoples' Region, on the west by Gera, on the north by Kersa, and on the east by Omo Nada districts. The altitude of the district ranges from 880 to 2400 meters above sea level. Perennial rivers include the Unat, Kawa, Waro and Offele.

A survey of the land in this woreda shows that 63.1% is arable or cultivable (38.4% was under annual crops), 13.6% pasture, 9.3% forest, and the remaining 14% is considered swampy, degraded or otherwise unusable. *Teff*, corn, vegetables and coffee are important cash crops of the district. Reports from different health centers in the district show that the district was malarious in the previous years. This study was conducted in Waro Kolobo kebele of the district in May and October 2018.

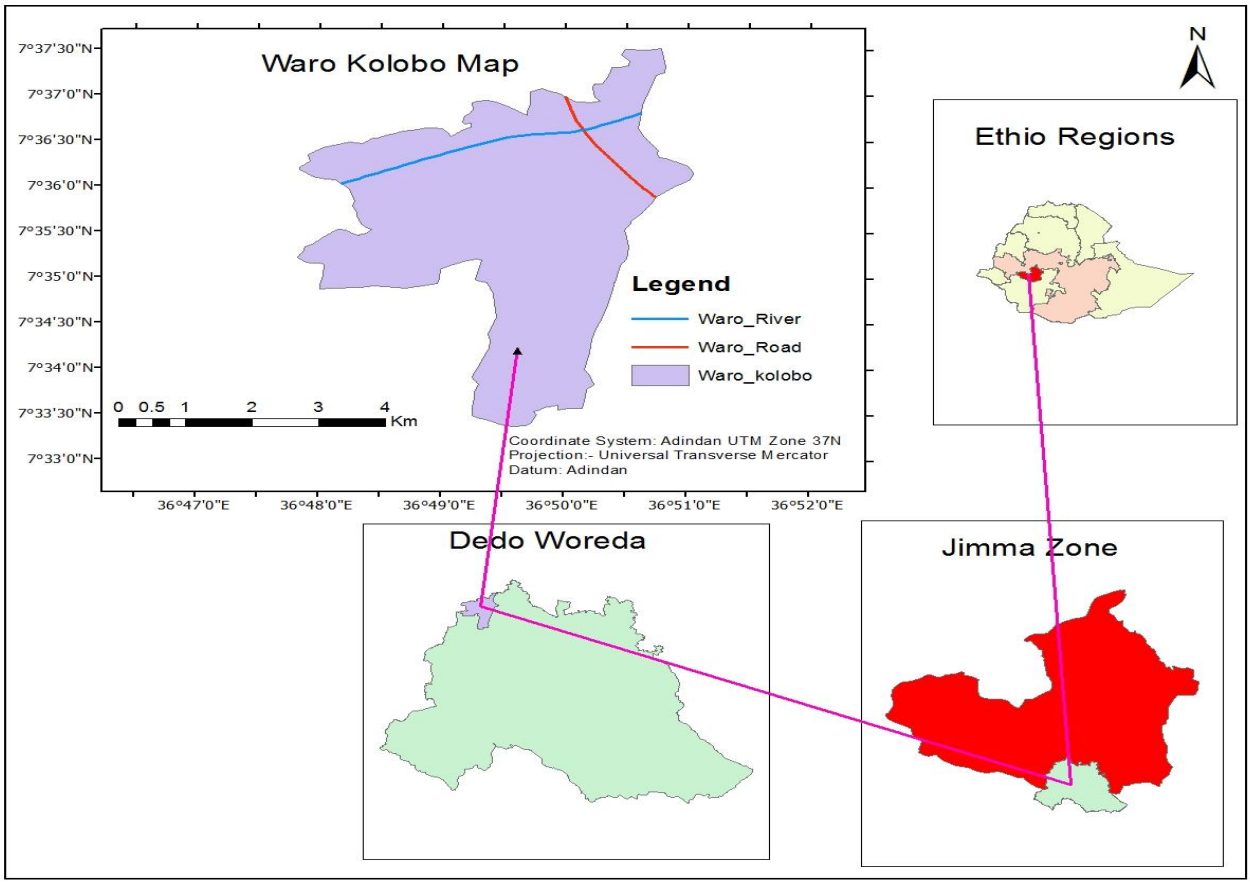

Figure 1*: Map of the study area*

Sources: Ethiopian map authority, 2018

4.2 Study design

Repeated community based cross-sectional study was employed

4.3 Source population

All residents in Waro Kolob kebele of Dedo district

4.4 Study population

All individuals within the selected households who took part in the study.

4.5 Inclusion criteria

Age ≥ 6months of both genders

Apparently healthy (defined as individuals with no complaints related to malaria symptoms)

Individuals residing in the village for more than one year

4.6 Exclusion criteria

Seriously ill patients who were not able to respond to the questionnaire

Individuals who were not voluntary to participate in the study

Individuals who were on anti-malarial treatment four weeks prior to data collection

4.7 Sample size

The sample size was calculated using the formula for single population proportion

$$n = \frac{(Z\ \alpha/2)^2 p(p-1)}{d^2}$$

n = the minimum sample size required for the study

Z $\alpha/2$= is the standard normal variable at 95 % confidence level (1.96)

p =prevalence of asymptomatic *Plasmodium* infection (6.3%)(33)

Since the total population was less than 10,000, the correction formula was applied.

nf=n/(1+n/N) = 362/ (1+362/6218) =342

Adding 10% non-response rate the final sample size was 376 individuals

4.8 Sampling technique

Out of 36 kebeles found in Dedo district, Waro Kolobo kebele was selected purposely due to its previous history of high malaria burden. The sampling frame of households (1269 HHs) were obtained from health post. 150 HHs (743 people) for the two cross-sectional surveys were selected by systematic random sampling. Taking an interval of "K" as 8 the first household was selected randomly. Finally, all individuals in the selected households those who were available during the data collection and fulfilling the inclusion criteria were included. After obtaining informed consent

and assent from parents/guardians, all members of the randomly selected households were requested to provide finger-prick blood samples.

4.9 Data collection method

4.9.1 socio-demographic data

Socio-demographic information and data on risk factors of malaria were collected using a pre-tested semi- structured questionnaire. The questionnaire contained information about socio-demographic characteristics of the study participants (age and sex), housing conditions, bed net uses and sharing of the living house with domestic animals. The questionnaire was first prepared in English and translated to *Afaan Oromo* which is the local language spoken by the residents of the kebele. The data were collected by laboratory professionals and health extension workers. To ensure maximum participation, HHs with absentees were revisited a second time on the same day to get those missing at the first visit

4.9.2. Blood sample collection and processing

Finger prick samples were collected from consenting individuals for blood film preparation and multi-species malaria rapid diagnostic test (RDT). The RDT CareStart™ combo test (Standard Diagnostic, Inc, Germany) were used in the study. The manufacturer's protocol was followed during the test. The RDT detects *Plasmodium falciparum* histidine-rich protein II (PfHRP-II) and other *Plasmodium* species *Plasmodium* lactate dehydrogenase (PLDH) for detection of *P. vivax, P. malariae* or *P. ovale*. The blood film was fixed with methanol on the field and transported to Jimma University Medical Parasitology laboratory. It was stained using 10% Giemsa solution for 15 minutes. The slides were examined using oil immersion objective by two experienced lab technologists independently. Any discordant result was resolved by a third technologist, who is blind to the earlier results. Each film was graded as positive (asexual malaria parasites seen) or negative (no malaria parasites seen) based on the examination of 200 fields of the thick smear.

4.9.3 Malaria Retrospective data collection

Five-year laboratory record of malaria from Offele health center, the health center utilized by Waro Kolobo residents, was also reviewed. The health center mainly uses microscopy for the diagnosis of malaria with RDTs also being used in cases of power interruption.

4.9.4 Study variables

Dependent variable: Asymptomatic *Plasmodium* infection

Independent variables:

Age

Sex

Educational status,

Occupational status,

Presence of eave,

Presence of hole in the wall

LLIN usage the previous night,

LLIN coverage,

Animals in the house & family size

4.9.5 Operational definition

Asymptomatic malaria: individuals having no symptom of malaria with positive blood film in Giemsa stained thick blood film.

Positive: Asexual malaria parasites seen based on the examination of 200 fields of the thick smear.

Negative: No malaria parasites seen based on the examination of 200 fields of the thick smear.

Household: all individuals living in the same house as family members.

Kebele: small administrative units in Ethiopia

Sufficient: when the ratio of the total LLIN owned by a household to the family members is at least 0.5 (assuming that one LLIN covers two individuals).

Not sufficient: when the ratio is less than 0.5

4.9.6 Data quality control

By discussing on tools before data collection, data were checked for completeness and consistency. Clean and grease free slides were used for blood film preparation to avoid scratch on the slides. First drop of blood was swiped off to decrease the effect of tissue fluids. Buffering tablet was used to adjust the PH of the giemsa solution. Positive and negative malaria control slides were tested to evaluate the performance of the staining solution. All CareStart^{TM} malaria test kits were labeled with participant ID number specifically given for this purpose and the test was done according to manufacturer's instruction. In all cases, the results of the CareStart test were determined earlier than microscopic results, with strict blinding to microscopic examination of the thick and thin blood

smears. Discordant slides between the microscopic readings were re-analyzed for the third time by the most experienced laboratory technologist from jimma university laboratory. All laboratory procedures were conducted based on the standard operating procedures.

The laboratory technologists who participated in blood film examination were blinded to RDT results. At least 10% of the negative slides and all the positive slides were randomly selected for internal quality control to be checked by another laboratory technologist.

4.9.7 Data analysis

Data were entered to Epi-data version 3.1 software and exported to SPSS version 20 for analysis. Descriptive statistics were utilized to summarize demographic profile of the study participants. Bivariate and multivariable logistic regression analyses were employed to determine the association between independent variables and the outcome/dependent variable. P-value ≤ 0.05 were considered as statistically significant. Long lasting insecticide nets coverage is said to be “sufficient” when the ratio of the total LLIN owned by a household to the family members is at least 0.5 (assuming that one LLIN covers two individuals), and “not sufficient” when the ratio is less than 0.5(34).

4.9.8 Ethical considerations

Ethical clearance was obtained from the institutional review board (IRB) of Jimma Institute of Health of Jimma University. Permission was sought from Jimma Town Health Office. Written informed consent and assent were obtained from each study participant and parents/guardians for children. During the survey, participants found positive were referred to Offele health center for treatment.

Chapter Five: Results

5.1 Socio-demographic characteristics of the study participants

Socio-demographic characteristics of the study participants and associated factors are presented in Table 1. In the two cross-sectional surveys conducted in May and October 2018, a total of 743 study participants were included. Mean age of the study participants was 20.706(SD ± 16.316) and 17.238(SD ± 13.8134) in the first and second survey, respectively. One hundred and seventy-seven (47.8%) and 203 (54.9%) of the study participants in the first and second surveys were males, respectively. All houses of the study participants were made of iron sheet of which almost all of them were sprayed in the last one year prior to both the first and the second survey.

Table 1. Socio-demographic characteristics of the study participants and associated factors in Dedo district, southwest Ethiopia

Characteristics		June 2018 survey, n (%)	October 2018 survey, n (%)
Age group (years)	0.5-15	188(50.8)	212(56.8)
	>15	182(49.20)	161(43.2)
Sex	Male	177(47.8)	204(54.7)
	Female	193(52.2)	169(45.3)
Educational status	Illiterate	140(37.8)	100(26.8)
	Literate	230(62.2)	273(73.2)
Occupational status	Farmers	70(18.9)	66(17.7)
	House wives	72(19.5)	42(11.3)
	Students	125(33.8)	170(45.6)
	Under age	92(24.9)	72(19.3)
	Others	11(3.0)	22(5.9)
Presence of eave	Present	275(74.3)	324(86.9)
	Absent	95(25.7)	49(13.1)
Presence of hole in the wall	Yes	231(62.4)	331(88.7)
	No	139(37.6)	42(11.3)
LLIN usage the preceding night	Yes	139(37.6)	259(69.4)
	No	231(62.4)	114(30.6)
LLIN coverage	Sufficient	246(66.5)	248(66.5)
	Not sufficient	124(33.5)	125(33.5)
Family size	<5	49(13.2)	4(1.1)
	≥5	321(86.8)	369(98.9)

5.2 Five-years trends of malaria

A retrospective study was conducted to determine the five years' trend of malaria by reviewing Offele health center health records and registers. Of 1,163 cases diagnosed for malaria, 35(3%) were positive for *Plasmodium*. Of which, 17(1.46%), 14(1.20%) and 4(0.34%) were positive for *P. vivax*, *P. falciparum* and mixed infections, respectively. The trend of malaria cases in Dedo district south-west, Ethiopia is presented in figure 2.

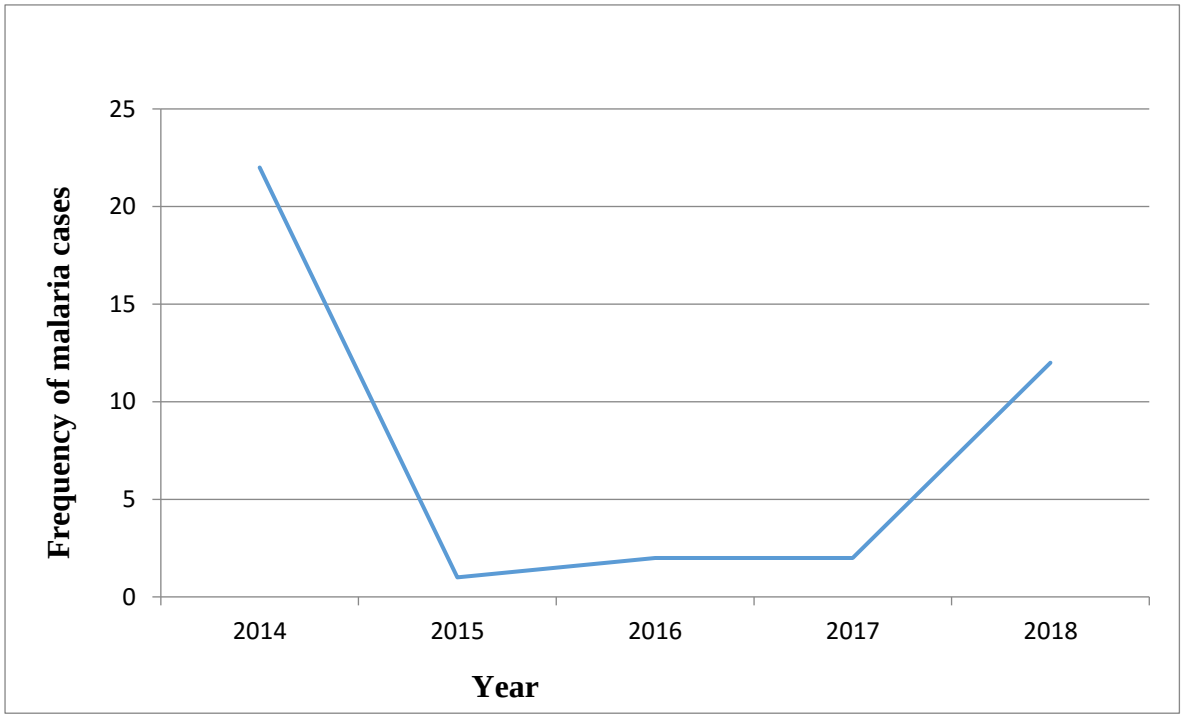

Figure 2: Trend of malaria cases at Offele Health Center (2014-2018)

5.3 Prevalence of malaria infection in the first survey

In the first survey, none of the study participants were positive for *Plasmodium* infection in both the microscopic examination and RDTs. Of the total 370 study participants in the first survey 65(17.6%) of them reported that they had history of malaria in the last one year. Multivariable analyses revealed that study participants of age greater than 15 years were more likely to get infected than participants with age range between 6 months and 15 years (AOR=2.353, 95% CI 1.307-4.237, p=0.004). Individuals found using LLINS last night before data collection were 3.5 times more likely to caught malaria the previous year (AOR=3.508,95% CI 1.964-6.266, p<0.001) and educational status (AOR=2.447, 95% 1.297-4.617, p=0.006) were significantly associated with self-reported previous one-year history of malaria. Table 2 showed factors associated with history of malaria in the first cross-sectional survey.

Table 2. Self-reported malaria and associated factors among the study participants in the first cross-sectional survey in Dedo district, southwest, Ethiopia

Characteristics		History of Malaria n (%)	Total n (%)	COR (95%CI)	AOR (95%CI)
Age group (years)	0.5-15	21(11.2)	188(50.8)	1	1
	>15	44(24.2)	182(49.2)	2.536(1.439-4.468)	2.353(1.307-4.237) *
Sex	Male	28(15.8)	177(47.8)	1	1
	Female	37(19.2)	193(52.2)	1.262(.736-2.165)	1.100(.618-1.958)
Educational status	Illiterate	38(27.1)	140(37.8)	2.801(1.620-4.844)	2.447(1.297-4.617) *
	Literate	27(11.7)	230(62.2)	1	1
LLIN usage the preceding night	Yes	24(10.4)	139(37.6)	1	1
	No	41(29.5)	231(62.4)	3.608(2.065-6.305)	3.508(1.964-6.266) *
LLIN coverage	sufficient	43(17.5)	246(66.5)	1	1
	Not sufficient	22(17.7)	124(33.5)	1.018(.578-1.794)	.837(.453-1.546)
Presence of eave	Present	49(17.8)	275(74.3)	1.071(.576-1.990)	1.045(.457-2.389)
	Absent	16(16.8)	95(25.7)	1	1
Presence of hole in the wall	Yes	41(17.7)	231(62.4)	1.034(.594-1.800)	1.071(.515-2.226)
	No	24(17.3)	139(37.6)	1	1
Animal kept in the house	Yes	56(18.7)	300(81.0)	1.556(.729-3.319)	1.976(.883-4.420)
	No	9(12.9)	70(19.0)	1	1
Family size	<5	10(20.4)	49(13.2)	1.240(.584-2.633)	1.166(.507-2.682)
	≥5	55(17.1)	321(86.8)	1	1

***significant at p < 0.05, 1=represents reference group**

5.4 Prevalence of malaria infection in the second survey

In the second cross-sectional survey, a total of 373 study participants were included of which 6(six) individuals were positive for *Plasmodium* and all cases were due to *P. vivax*. However, none of the study participants were positive by the RDT test. In this survey, 43(11.5%) of the study participants reported that they had malaria in the previous one year. Table 3 shows factors associated with self-reported of malaria in the second survey.

Table 3. Self-reported malaria and associated factors among the study participants in the second cross-sectional survey

Characteristics		History of malaria n (%)	Total n (%)	COR (95%CI)	AOR (95%CI)
Age (years)	0.5-15	19(9.0)	212(56.8)	1	1
	>15	24(14.9)	161(43.2)	1.779(.938-3.376)	1.736(.909-3.314)
Sex	Male	20(9.8)	204(54.7)	1	1
	Female	23(13.6)	169(45.3)	1.449(.766-2.741)	1.479(.762-2.867)
Educational status	Illiterate	15(15.0)	100(26.8)	1.544(.787-3.029)	1.012(.428-2.393)
	Literate	28(10.3)	273(73.2)	1	1
LLIN usage the preceding night	Yes	28(10.8)	259(69.4)	1	1
	No	15(13.2)	114(30.6)	1.250(.640-2.442)	1.249(.623-2. 506)
LLIN coverage	sufficient	26(10.5)	248(66.5)	1	1
	Not sufficient	17(13.6)	125(33.5)	1.344(.699-2.583)	1.313(.657-2.624)
Presence of eave	Present	38(11.7)	324(86.9)	1.169(.437-3.131)	.855(.092-7.976)
	Absent	5(10.2)	49(13.1)	1	1
Presence of hole in the wall	Yes	39(11.8)	331(88.7)	1.269(.430-3.748)	1.189(.100-14.155)
	No	4(9.5)	42(11.3)	1	1
Animals kept in the house	Yes	24(10.2)	236(63.3)	1	1
	No	19(13.9)	137(36.7)	1.422(.748-2.704)	1.340(.670-2.680)
Family size	<5	1(25.0)	4(1.1)	2.595(.264-25.522)	2.348(.233-23.628)
	≥5	42(11.4)	369(98.9)	1	1

1= represents reference group

Chapter six: Discussion

A five-year malaria trend analysis in offele health center, a health facility used by Waro Kolobo residents showed that out of the total 1163 patients that were diagnosed for malaria at the Health Center only 35 patients were tested positive for malaria and proportion of cases due to *P. falciparum* and *P. vivax* was equal. Given that these are febrile cases seeking treatment at the Health Center, the prevalence of malaria was low in the area. The five-year trend appeared to show a sharp decline in 2015 (only two malaria cases were recorded) however, a slight increase was observed with (12/1163) malaria cases in 2018. However, as the data were obtained from one health center only and the possibility that some of the residents might have visited private health facilities for treatment of fever, conclusion based on this finding alone may not be plausible. Moreover, as information of the patients recorded in the health center is limited to demographic profile only, similar to all public health facilities in Ethiopia, history of travel before presenting themselves to the health facility could not be obtained. Travel to malaria endemic areas may be an important risk factor in areas of low malaria transmission (35, 36).

The prevalence of asymptomatic *Plasmodium* infection in the area is also reflected in the two cross-sectional surveys in this study. In the first cross-sectional survey, none of the study participants were positive for malaria while in the second cross-sectional survey, 1.6% of them were positive. Given that the first and second surveys were conducted in the minor and major malaria transmission season of the area, respectively, low burden of malaria is evident in the area.

However, prevalence of asymptomatic *Plasmodium* infection in the study area may have implication on the ongoing malaria elimination efforts in Ethiopia as those cases can be reservoirs of infection in the study setting. It should also be noted that in this study the methods used to detect the *Plasmodium* species were microscopy and RDT, which are less sensitive compared to molecular methods (37). Therefore, prevalence of asymptomatic *Plasmodium* infection could be higher than recorded from the study area.

The prevalence of asymptomatic *Plasmodium* infection from blood film microscopy examination was (1.6%) which is lower than study in Kenyan coast (2%)(38), Temotu province, Solomon Islands (2.7%)(39) and West Arsi Ethiopia (5%)(40). But the result of this study is higher when compared to the study conducted in Iranshahr district of southeastern Iran (0.6%)(41). The low prevalence of malaria cases in our study area could be due to the effectiveness of the control measures being implemented in the area including IRS usage.

A recent study done in Jimma Town also reported low prevalence of asymptomatic *Plasmodium* infection (42). Malaria control activities based on distribution of LLINs, annual application of IRS and case detection and treatment has been intensified in Jimma area in the last decade, as in most parts of the country. The low prevalence and declining trend of malaria in this study and the other studies done in the area could be the result of these scaled up control interventions. Concerted effort is required to further intensify the control activities to move to the pre-elimination stage.

The observed 1.6% asymptomatic *Plasmodium* infection by microscopy while negative using RDT could be due to the fact that performance of RDTs may be affected by factors including storage temperature, especially during transportation from place of manufacturing or supply to final place of use, suboptimal sensitivity at low parasite densities and exposure to adverse environmental conditions during distribution (43). Both microscopy and RDTs are known to produce negative results when parasitemia levels are below their limits of detection, 5-10 and 100 parasites/μL blood, respectively (44, 45).

In most studies, microscopy showed better performance in detecting malaria parasites than RDTs. In a study conducted in Nigeria in 2016, microscopy detected (85.7%) of malaria parasites which is (68.8%) for RDT(46). Another study in Burundi showed that 70.5% and 48.4% of the study participants were positive by microscopy and RDT, respectively (47). Moreover, a study on assessment of microscopy and RDT showed detection rate of 66.8% and 36.8%, respectively (48).

In the first cross-sectional survey, age, educational status and LLINs use were significant predictors of self-reported malaria in the preceding year. The study revealed almost all of households owned at least one bed net, but only 62.4% of them used the LLINs the previous night before the survey. A comparable use of LLINs (68.3%) was reported in a study from Limmu Seka District of Jimma Zone, Ethiopia (49). Similar to the first survey, in the second survey all of households owned at least one LLIN, with 69.4 % of the participants used the LLINs the night before the survey. This calls for interventions on human behavior to effectively utilize the LLINs for malaria prevention. In some studies in Africa, higher proportion of individuals using the LLINs were reported (50). Improving the use of LLINs is critically required as high coverage and use has a herd effect whereby the larger proportion of individuals utilizing the LLINs could be protected from malaria infection (51).

Chapter seven: Conclusion and Recommendation

7.1 Conclusion

Malaria trend analysis in the study area showed that the disease is still causing significant problems. Our results from microscopic examination show that prevalence of asymptomatic *plasmodium* infection in the study area was low (6/373). But it could contribute to the ongoing malaria elimination efforts in Ethiopia as those cases can be reservoirs of infection in the study area. Microscopy and RDT were the methods used to detect the *Plasmodium* species, though they are less sensitive compared to molecular methods. LLINs coverage per household was high while LLINs utilization was modest in both surveys. Educational status and LLINs usage appear to influence history of malaria infection in the preceding one year.

7.2 Recommendation

Although microscopy and RDT detected considerable numbers of asymptomatic infections in apparently healthy individuals, the use of a highly sensitive molecular diagnostic methods like PCR offers a more accurate assessment of the magnitude of asymptomatic infections. Hence, further studies are needed for a better understanding of the asymptomatic *Plasmodium* infections and their contribution to the dynamics of malaria transmission. Awareness creation on proper utilization of LLINs are required for the study participants as LLINs are important in protecting indoor and night biting mosquitoes.

Declaration

I, the undersigned, declare that this thesis is my own original work and it has not been presented within the same organization or in other universities, colleges or other institutions for similar degree or other purpose.

I agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the Institute of health in effect at the time of grant is forwarded as the result of this application.

Name of the student	Signature	Date
Tadesse Duguma (BSc, MLT)	_____	_____

Approval of the Advisors

This thesis has been submitted with our approval as University advisors.

1. Prof. Delenasaw Yewhalaw (PhD)

   Signature _____ Date _____

2. Mr. Endalew Zemene (MSc, PhD fellow)

   Signature _____ Date _____

Approval of the Internal Examiner

This thesis has been submitted with my approval as University examiner.

Name of Internal Examiner

1. Mr. Abdisa Hordofa (MSc, PhD fellow)

   Signature _____Date_____

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Annexes

Annex 1: Information Sheet

Name of the Principal Investigator*:* Tadesse Dhuguma

Name of the organization: Jimma University

Introduction: This information sheet is prepared by groups of researchers whose main aim is to study asymptomatic malaria infection and determine blood meal sources of malaria vectors.

Purpose:

The purpose of this research is to determine asymptomatic malaria infection and analyze the blood meal sources of malaria vectors. Malaria results in significant morbidity and socio-economic burden in Ethiopia.

Several epidemiological studies have been conducted in Ethiopia, however, these studies are often based on health facilities and the possible asymptomatic carriage in household members has not been well addressed so far. Therefore, considering this, we have planned to undertake this research

Procedure:

We kindly invite you to take part in this project which is aimed at determining magnitude of asymptomatic infection among individual members of a household. If you are willing to participate in this project, you need to understand and sign the agreement form. For laboratory examination, you will provide blood sample from your finger. The blood samples will be collected following a standard protocol. The laboratory examination results will be kept confidential using coding system whereby no one will have access to your laboratory results. If the result of the laboratory examination shows positive, this will only be communicated to the health professional for management of the case.

Benefits

If you participate in this research, you may get direct benefit that the test result will be used for the purpose of management of your health. In addition, your participation will help us in studying the magnitude of asymptomatic infection in the area, which is an input for national malaria control activities being carried out

Incentives

You will not be provided any incentives to take part in this research.

Confidentiality:

The information that we collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. It will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and the concerned health professional.

Right to refuse or withdraw

You have full right to refuse from participating in this research if you do not wish to participate; and this will not compromise the health services you get at the health institutions in any way at any time.

Whom to contact

If you have any questions, contact any of the following two individuals and you may ask at any time you want:

1. Ato Endalew Zemene - Jimma University, college of public health and Medical sciences, Department of Medical Laboratory Sciences and Pathology**,** Jimma, Ethiopia

Telephone no: 0912071295

2. Ato Tadesse Dhuguma- Jimma University, college of public health and Medical

Annex 2: Consent form

Introduction

We are asking you to take part in a research study on asymptomatic malaria infection and analysis of blood meal sources of malaria vectors at Dedo district, Jimma zone, south west Ethiopia.

We want to be sure that you understand the purpose and your responsibilities in the research before you decide if you want to be part of the study. Please ask us to explain any words or information that you may not understand.

Information about the Research

This is a research study that would involve collection of about 2ml of blood from a peripheral vein for the detection of *plasmodium* parasitaemia.

Possible Risks (explain risks –blood collection)

It is very unlikely that participation in this research will expose you to any physical, social or psychological risks.

Possible Benefits

Participation in this research may not benefit you directly. But results from this study will be used to inform decisions in implementation and strengthening of programs aimed at controlling and eradicating malaria in Ethiopia.

If You Decide Not to Be in the Research

You are free to decide if you want to be part of this research or not.

Confidentiality

We will protect information about you taking part in this research to the best of our ability. We will neither use your name in any reports nor discuss your participation with anyone outside the research team.

Payment

No payments will be made for participation.

Leaving the Research

You may end your participation at any time with no negative consequence to you.

If You Have Questions about the Study

If you have any questions about the research, you can call at 0912071295 (Endalew Zemene)

or 0917396437 (Tadesse Dhuguma)

Your rights as a Participant

This research will be reviewed and approved by the Jimma University ethical review board and permission to conduct the research will be received from the jimma zone Health office. If you have any questions about how you are being treated by the study or your rights as a participant, you may contact

1. Ato Endalew Zemene - Jimma University, college of public health and Medical sciences, Department of Medical Laboratory Sciences and Pathology**,** Jimma, Ethiopia

Telephone no: 0912071295

2. Ato Tadesse Dhuguma- Jimma University, college of public health and Medical sciences, Department of Medical Laboratory Sciences and Pathology**,** Jimma, Ethiopia

Telephone no: 0917396437

አባሪ 2፡ የስምምነት ፎርም መግቢያ

በደዶ ወረዳ ጅማ ዞን ዉስጥ ምልክት የለሽ የወባ በሽታ መስፋፋትን በተመለከተ በምናደርገው ጥናት ላይ እንድሳተፉ በትህትና እንጠይቃለን። የ ጥናቱ አካል ከመሆንዎ በፊት የጥናቱን አላማ እና የሚጠበቅቦትን በደንብ እንድረዱ እንፈልጋለን። እባክዎ ግልጺ እንድሆንሎት የሚፈልጉትን ሃሳብ ይጠይቁን።

ስለ ጥናቱ መረጃ

ይህ ጥናት የወባ በሽታ አምጭ ጥገኛ ተህዋስን ለመለየት ስባል ከጥናቱ ተሳታፊ ሰው ደም ስር ሁለት ሚሊ ሊትር ደም መዉሰድን ያካትታል።

በጥናቱ በመሳተፎ ልያጋጥም የሚችል አደጋ ወይም ስጋት

በዚህ ጥናት በመሳተፎ የሚገጥሞዎት ወይም ልደርስቦዎት የሚችል አካላዊ፣ማበራዊም ሆነ አምሮዊ ችግር የለም።

ከጥናቱ ልገኝ የሚችል ጥቅም

በዚህ ጥናት በመሳተፎ ቀጥተኛ ጥቅም ባያገኙም ከጥናቱ የምገኘው መረጃ ወባን ለመከላከል፣ለመቆጣጠርም ሆነ ብሎም ከሃገርቱ ለማጥፋት የመረጃ ግበት ይሆናል።

በጥናቱ ላለመቀጠል ከወሰኑ

በጥናቱ ላለመቀጠል ከፈለጉ በየትኛውም ግዘ ማቆም ይችላሉ። ምስጥርን ስለመጠበቅ

ምስጥሮዎን ለመጠበቅ ስባል በዚህ ጥናት መሳተፎዎንም ሆነ ስሞዎን ለጥናቱ አባላት ካልሆነ በቀር ለለላ ወገን ይፋ አይደረግም።

ክፍያን በተመለከተ

ለተሳትፎዎ ሲባል የሚፈጸም ምንም አይነት ክፍያ የለም። ጥናቱን በተመለከተ ጥያቀ ካሎት

ጥናቱን በተመለከተ ጥያቀ ካሎት በተከታዮቹ የስልክ ቁጥሮች ማግኘት ይችላሉ፣ እንዳለው ፣ዘመነ፡ 0912071295 እና ታደሰ፣ዱጉማ፡ 0917396437

እንደተሳታፊ ያሎት መብት

ይህ ጥናት ከመጀመሩ በፊት ከጅማ ዩኒቨርሲቲ የጥናት እና ምርምር ኮሞተ ፈቃድ የምጠየቅ እና በጽሁፍ የምወሰድ ይሆናል።

በጥናቱ መሀከል ለሚያጋጥሙ የ አያያዝ እና ለሎች ችግሮች ጥያቀ ካሎት በተከታዮቹ የስልክ ቁጥሮች ማግኘት ይችላሉ፣ እንዳለው ፣ዘመነ፡ 0912071295 (የጅማ ዩኒቨርሲቲ የላቦራቶሪ ትምህርት ክፍል መምህር)

ታደሰ፣ዱጉማ፡ 0917396437 (የጅማ ዩኒቨርሲቲ የላቦራቶሪ ትምህርት ክፍል ተማር)

Guca 2: Boca waligaltee

Yeroo ammaa kana qorannoo dhibee busaa mallattoowwan hinqabine irratti kaumsa dhukkuba busaa waliqabatee kibba lixa Itiyoophiyaa, Godina jimma, Aanaa Dedootti geggeessaa waan jirruuf isinis kana hubachuun kana keessatti akka gooda fudhattan kabajaan isin gaafanna. Qorannoo gaggeeffamu keessatti osoo hin hirmaatin dura faayidaa qorannichaa fi ga`e isin irraa eegamu irratti gaaffii fi yaada qabdan akkassumas dhimmoota isiniif hingalle irratti gaafattanii hubannoo keessan cimsachuu akka dandeessan isiniif mirkaneessuu barbaanna.

Odeeffannoo waliigalaa Qorannichaa

Qorannoon kun mallattoowwan dhibee busaa adda baasuuf sakattainsi dhigaa kan gaggeeffamu ta`a. Qorannicha keessatti hirmaachuun miidhama qaamaa, hawaasumaafi sammuu kamiyyuu qaqqabsiisuu akka hin dandeenye isiniif mirkaneessuu barbaanna.

Bua`a qorannichaa irraa kallattin fayyadamaa tauu baattus sagantaawwan ittisaa fi to`annoo dhibee busaa akka biyyaatti hojiirra ooluu jajjabeessuufi fayyadamaa isin taasisa. Qorannicha keessatti qooda fudhachuunfi ykn dhisuuf mirga guutuu qabda.

Odeeffannoo dhunfaa nama qorannicha keessatti hirmaatee qaama biraaf kennuu fi maqaa nama

Sanaa gabaasa kamuu keessatti hindhiyaatu. Hirmaannaan taasifamu kaffaltii hin qabu. Gaaffiifi yaada yoo qabaattan teessoo fi bilbilli keenya kan armaan gaditi

1. Obbo Indaalew Zemane- 0912071295
2. Obbo Taaddassee Dhugumaa- 0917396437

Teessoo- Yuniversiti jimma, departmanti medikala laboratoorii saayissi irraa.

Annex 3: English Questionnaire

Dear Sir/madam;

My name is_____

I am Master's Degree students from Jimma University. Thus this questionnaire is prepared to get appropriate information on the prevalence of asymptomatic malaria.

The information that I will obtain using this questionnaire will be used only for research purpose and also I need to assure you that confidentiality is our main quality.

Therefore; I politely request your cooperation to participate in this interview. You do have the right not to respond at all or to withdraw in the meantime, but your input has great value for the success of our objective

Did you agree _____

Did not agree _____

Thank you for your cooperation!!!

Part I: - Background information

1. Zone (gote)/ village _____
2. House No_____
3. Household Code: _____
4. Head of the house hold 1. Male 2. female
5. Respondent position in the house holds _____

Part II Socio-demographic data

No	Questions	Possible choices/Answers	Remark
1	Sex of the respondent	1. male 2. female	
2	Age of the respondent	_____in year	
3	Educational status	1. Illiterate 2. 1st cycle (1-4) 3. 2nd cycle (8-10) 4. Secondary (9-10)	
4	Hojii	1. Hojjetaa mootumma 2. Qonnan bulaa 3. Daldalaa 4. Hoji guyyaa	
5	Baayina maatii	1.umurii 5 gadi_____ 2. dubartii ulfaa 3. Waliigala	

Part III Housing Condition

No	Questions	Possible choices/Answers	Remark
6	Main material of the roof	1. Thatched 2. Corrugated iron sheet 3. Others_____	
7	Main material of the floor	1. Earthen 2. Cemented 3. Wooden 4. Other, specify_____	
8	Cleanliness of the house	1. Good 2. Medium 3. Poor	
9	Ventilation of the house	1. Good 2. Medium 3. Poor	
10	illumination of the house	1. Good 2. Medium 3. Poor	
11	Distance from vector breeding site	_____in meters	
12	Are there animals in the house?	1. Yes 2. No	
13	If yes, for Q 12 which animal	1. Dog 2. Cat 3. Ruminant animal 4. Non Ruminant animal	
14	Is there any of your family member? experienced malaria in the last 1	1. Yes 2. No_____	
15	If yes who was affected?	1. Pregnant woman **2.** Under five	
16	Is he or she get treatment?	1.Yes 2. 2.No	
17	If the answer for Q 16 is yes where?	1. HP 2. HC 3. Hospital	

18	If the answer for Q 16 is No why?	1. No health facility in the area 2. Can`t afford to buy the drug	
19	Is there any stagnant water in your compound or surrounding?	1. Yes **2.** No	
20	Is there chemical spraying to control mosquitoes?	1. Yes **2.** No	
21	If yes, how frequent?	1. Once in a year 2. twice and more in a year	
22	Is there impregnated mosquito net in your household?	1. Yes 2. No	
23	How many ITN do you have?	1. _____	
24	Is the family use impregnated mosquito net?	1. Yes 2. No	
25	Uses ITN last night	1. Yes 2. No	
26	If yes Q 25 which family members	1. all family members 2. only father and	
27	If yes how frequently do you use it?	1. Always 2. Sometimes 3. None	
28	Observe the properness of the ITN Utilization	1. Proper 2. Fair 3. Improper	

አባሪ3: ቃለ-መጠይቅ

ውድ ጌታ / እመቤት;

የ እኔ ስም_____ ነዉ።

እኔ የጅማ ዩኒቨርሲቲ ማስተር ዲግሪ ተማሪ ነኝ። ይህ መጠይቅ የወባ በሽታ መስፋፋት በተመለከተ ተገቢውን መረጃ ለማግኘት ነው።እኔ ይህን መጠይቅ በመጠቀም የማገኘዉ መረጃ ለዚህ ምርምር ዓላማ ብቻ የሚዉል ይሆናል፤ እንዲሁም ሚስጢራዊነቱም የተጠበቀ እንደሆነ ላረጋግጥላችሁ እወዳለሁ። ስለዚህ፤ በዚህ ቃለ መጠይቅ ላይ ለመሳተፍ ትብብርዎን እየጠየኩ ትክክለኛው ምላሽዎ ለዚህ ጥናት ስኬት ታላቅ ዋጋ አለዉ።

ነገር ግን ለቃለመጠይቁ በከፍልም ሆነ በሙሉ መልስ ያለመስጠት መብትዎ የተጠበቀ ስሆን ቃለመጠይቁን

ለማቆረጥ ከፈለጉም በየትኛዉም ግዘ ይችላሉ።

ይስማማለሁ? _____ አይስማሙም? ---------- ለ ትብብርዎ እናመሰግናለን።

ክፍል አንድ: - የዳራ መረጃ

1. ዞን (ጎጥ) / መንደር _____
2. ቤት ቁጥር____
3. የቤት ኮድ: ___
4. የቤተሰብ ሃላፊ 1. ወንድ 2. ሴት
5. የጥናቱ ተሳታፊ ሃላፊነት

ክፍል **II** ማህበራዊና-ሕዝብ አወቃቀር ውሂብ

ተ.ቁ	ጥያቄዎች	ሊሆኑ የሚችሉ ምርጫዎች / መልሶች	ማመልከቻ
1	የምላሽ ሰጪ ጾታ	1. ተባዕት 2. ሴት	
2	የምላሽ ሰጪ እድሜ	ዓመት	
3	የትምህርት ሁኔታ	1. ምንም ያልተማሩ 2. 1 ደረጃ (1-4) 3. 2 ደረጃ (8-10) 4. የሁለተኛ ደረጃ (9-10)	
4	ሞያ	1. መንግስታዊ ሰራተኛ 2. አርሶ አደር 3. ነጋዴ 4. የቤት እመቤት 5. ዕለታዊ ሥራ የምሰራ/የሚትሰራ 6. ተማሪ 8. ስራ አጥ 9. ሌሎች	
5	የቤተሰብ መጠን	1. ከ5 አመት በታች___ 2. እርጉዝ 3. ለሎች---------- 4. ድምር_____	

ክፍል **III** የመኖሪያ ቤት ሁኔታ

	ጥያቄዎች	ሊሆኑ የሚችሉ ምርጫዎች / መልሶች	አመለከተ
6	ጣሪያዉ የተሰራበት ቁስ	1. ሣር 2. ቆርቆሮ 3. ሌሎች, ካሉ ይገለጽ_____	
7	ወለሉ የተሰራበት ቁስ	1. ሸክላ 2. ሊሾ 3. የእንጨት 4. ሌሎች, ካሉ	
8	የቤት ንጽሕና	1. ጥሩ 2. መካከለኛ 3. ደካማ	
9	የቤት ዉስጥ የአየር ዝዉዉር	1. ጥሩ 2. መካከለኛ 3. ደካማ	
10	ቤት የምገባ የ ብርሃን መጠን	1. ጥሩ 2. መካከለኛ 3. ድኻ	
11	የወባ ትንኝ መራቢያ ስፍራ ከቤት ያለዉ ርቀት በሜትር	----------ሜትር	
12	ቤት ውስጥ እንስሳት አሉ?	1. አዎ 2. አይ	
13	ለጥያቄ 12 መልስዎ አዎ ከሆነ የትኛው እንስሳ ነው ?	1. ውሻ 2. ድመት 3. አመንዣኪ እንስሳ 4. አመንዣኪ ያልሆነ	
14	ባለፉው 1 ወር ውስጥ በወባ የተያዘ የቤተሰብ አባል አለ?	1. አዎ 2. አይ	
15	አዎ ከሆነ ማን ነው?	1. እርጉዝ ሴት 2. ከአምስትአመት በታች የሆኑ ህጻናት 3. ሌሎች----	
16	ህክምና አግኝተዋል?	1.አዎ 2 .አይ	
17	ለጥያቄ 16 መልስዎ አዎ	1. ጤና ከላ 2. ጤና ጣቢያ 3. ሆስፒታል	
18	ለጥያቄ 16 መልስዎ አይ ከሆነ?	1. በአካባቢው የጤና ተቋም የለም 2. የመግዛት አቅም የለኝም	
19	የእርስዎ ግቢ ዉስጥ ወይም	1. አዎ	
20	ትንኞች ለመቆጣጠር	1. አዎ 2. የለም	

21	አዎ, ካልክ ለስንት ግዘ ነው?	1. በዓመት አንድ ግዘ 2. 2. ሁለት ጊዜ እና ከዚያ በላይ በዓመት	
22	የእናንተ ቤት ውስጥ አጎበር	1. አዎ አይ	
23	ስንት ኬሚካል የተነከረ	1.	
24	ቤተሰብዎ ኬሚካል የተነከረ	1. አዎ 2. አይ	
25	ትናንት ማታ አጎበር	1. አዎ 2. አይ	
26	ለጥያቄ 25 መልስዎ አዎ	1. ሁሉም የቤተሰብ አባላት 2. አባት እና እናት ብቻ 3. ልጆች ብቻ	
27	መልስዎ አዎ ከሆነ ለምን	1. ሁል ጊዜ 2. አንዳንድ ጊዜ	
28	የ አ ጎ በ ር አ ጠ ቃ ቀ ም	1 . በ ጣ ም ጥሩ 2 . ጥሩ 3. ተ ገ ቢ ያ ል ሆ ነ	

Guca 3- Gaafannoo

Obbo/Aadde----------

Maqaan koo----------nin jedhama, barataa digirii 2ffaa yuuniversitii jimmaati yeroo ammaa kana qorannoo mallattoowusan dhibee busaa irratti gaggeessuuf odeeffannoowwan nabarbaachisan argachuuf

gaafannoowwan adda addaa qopheessuun ragbseera. Odeeffannoon argamus

bu`aa qorannoo mirkaneessun qofaafi waanta`eef atoomni isin nuuf taasiftan bu` aa qorannichaaf iddoo guddaa waan qabuuf deggersa cimaa akka nuuf

taasistan kabajaan isin gaafanna.

Irratti waliif galeera---------- Irratti walii hin galu--------

---------- Atooma keessaniif galatooma!

Kutaa 1- odeeffannoo waliigalaa gaafataman

1. Ganda----------zoonii gandaa----------garee----------
2. Lakkoofsa manaa----------
3. Koodii maatii----------
4. Maatii qaama hoogganu: A. Dhira B. Dubara
5. Ga`ee gaafatamaa maati keesssatti qabu----------

Kutaa 2: Ragaa haala hawaasummaa ibsu

lakk	Gaaffii	Filannoo	yaada
1	Saala gaafatamaa	1.Dhiira 2. Dubara	
2	umurii	wagga_____	
3	Sadarkaa barumsaa	1. Hinbaranne 2. kutaa (1-4) 3. kutaa (8-10) 4. kutaa (9-10) 5. kutaa (11-12)	
4	Hojii	1. Hojjetaa mootumma 2. Qonnan bulaa 3. Daldalaa 4. Hoji guyyaa	
5	Baayina maatii	1.umurii 5 gadi_____ 2. dubartii ulfaa 3. Waliigala	

Kutaa 3: Haala manaa

Lakk	Gaaffi	Filannoo _____	yaada
6	Guutun mana kan ijaarame	1.citaa 2. qorqorroo 3. kan biro yoo _____ jirate	
7	Lafa jala manichai irraa	1. biyyee 2. simmintoo 3. xawulaa mukaa 3. Kan birroo	
8	Qulqullina manichaa	1.gaarii 2. giddu galeessa 3. gad aanaa	
9	Hala manicha itti qilleensa galchu	1. gaarii 2. giddu galeessa 3. gad aanaa	
10	Haala manichii itti ifa galchu	1. gaarii 2. giddu galeessa 3. gad aanaa	
11	Fageenya iddoo wal hormaata bookee busaa irra qabu	Meetira	

12	Beeladoonni manatti galan jiruu?	1.eeyyee 2. Lakki	
13	Deebiin gaaffii 12 eeyyee yoo	1 oola 2 . Rea'e 3. Beeladoota---------	
14	Maatii kee keessa ji`a tokkoon as namnii dhukkuba bussan qabame jiraa	1 eeyyee 2. lakki_____	
15	Yoo deebiin gaaffii 14 eeyyee ta`eenyuu?	1. Dubarti ulfaa 2. Ijoollee umurii 5 gadii 3. Kan biroo	
16	Namni qabame yoo jiraate yaalameeraa?	1.eeyyee 2. lakkii	
17	Deebiin gaaffii 16 eeyyee yoo	1. kellaa fayyaa 2. buufata fayyaa 3. Hospitaala	
18	Deebiin gaaffii 16 lakki yoo ta`e	1.Iddoon itti yaalaman dhiyeenna waan hin jirrreef 2.Qoricha bitachuuf mallaqa waan hin qabneef	Yaada
19	Bishaan ciisu dallaakee keessa	1.Eeyyee 2. Lakki	

20	Keemikaalli bookee busaa ittisuf biifamuu jiraa?	1.Eeyyee 2 . Lakki	
21	Deebiin gaaffii 20 eeyyee yoo	1.Waggaatti yeroo tokko 2. Waggaatti yeroo lamaafi isaa ol	
22	Saaphana siree bookee busaa mana kee jiraa	1.eeyyee 2. lakki	
23	Saaphana siree bookee busaa hagam qabda?	1._____	
24	Maatiin kee saaphana siree bookee busaa ni fayyadamu?	1.Eeyyee 2. Lakki	

25	Halkan darbe saaphana siree bookee busaa	1.Eeyyee 2 . Lakki	
26	Deebiin gaaffii 25 eyyee yoo t a`e eenyutu fayyadame?	1.Miseensa maatii hunda 2. Abbaa fi haadha mana qofa 3. Daaiimman qofa	
27	Deebiin gaaffii 26 eeyyee yoo	1.Yeroo hunda 2. Darbee darbee 3. Hinfayyadammu	
28	Haalli itti fayyadama saaphana	1. Sirriidha 2. Madaalawaadha 3. Sirriimiti	

Annex 4: Procedure for blood film examination

DESCRIPTION OF ACTIVITY
4.1. Examining the thick film 1. Place the Giemsa-stained blood film to be examined on the microscope stage, with the label to the left. Position the thick film in line with the 10x objective lens. 2. Switch on the microscope, adjust the light source optimally and find the focus by looking through the ocular and the 10x objective. 3. Scan the blood film for parasites and blood elements. Select part of the film that is well stained and has evenly distributed white blood cells. 4. Place a small drop of immersion oil on the thick film. To avoid cross-contamination, ensure that the immersion oil applicator never touches the slide. Do not allow the 40x objective to touch the oil. 5. Switch the 100x oil immersion objective over the selected portion of the thick film. Use

DESCRIPTION OF ACTIVITY
7. Examine the slide in a systematic manner. Start at the top left of the film (marked with a vertical green arrow on **Fig. 1**) and begin at the periphery of the field, then move horizontally to the right, field by field. 8. When the other end of the film is reached, move the slide slightly downwards, then to the left, field by field, and so forth (see below). For efficient examination, continuously focus and refocus with the fine adjustment throughout examination of each field.

MM–SOP–08: MICROSCOPY EXAMINATION OF THICK AND THIN BLOOD FILMS FOR IDENTIFICATION OF MALARIA PARASITES

FLOW CHART	DESCRIPTION OF ACTIVITY
4.2. Determining whether a thick film contains malaria parasites and identifying the species 1. Examine the thick film under the oil immersion objective, field by field, horizontally or vertically. 2. Read a minimum of 100 fields before declaring that no malaria parasites were seen. 3. If parasites are found, scan additional 100 fields to increase the chance of identifying mixed infections.	**4.2. Determining whether a thick film contains malaria parasites and identifying the species** 1. Continue to examine the slide for 100 high power or oil immersion fields. Move the blood film by one high-power field each time, following the pattern. Use the fine adjustment to focus. 2. A minimum of 100 high-power fields must be examined before a thick film can be declared as having "no malaria parasites seen". If possible, the whole thick film should be scanned.

3. If parasites are observed, a further 100 fields must be examined before final identification of the species, ensuring that a mixed infection is not overlooked.

4. The thin blood film should always be examined to identify parasite species definitively. The thin film allows visualization of parasite and red cell morphology, unlike

the thick film. Perform an examination at the feathery end or edge of the thin film, as described in procedure 4.3 below.

5. Identify and record all species and stages observed in the malaria microscopy blood register. See MM-SOP 6b: Recording and reporting microscopy results.

Note: Refer to the WHO bench aids for the diagnosis of malaria for identification of each species

FLOW CHART	DESCRIPTION OF ACTIVITY
4.3. Examining the thin film to confirm Species and mixed infections 1. The thin blood film must be examined to confirm species and mixed infections. 2. Place a drop of oil on the feathery edge of the film. 3. Move from the 10x lens to the 100x oil immersion lens, and focus on the thin film. 4. Read the thin or feathery edge of the film, moving from one field to the next, horizontally or vertically 5. Scan the film until all the species have been confirmed and there is minimal overlap. Follow the pattern of movement shown in Fig. 2. Move along the edge of the film, then move the slide outwards by one field, inwards by one field, returning in a lateral movement and so on. 5. Continue examining the thin film until the presence and species of malaria parasites have been confirmed. Identify and record all species and stages observed in the malaria microscopy blood register. See MM- SOP 6b: Recording and reporting microscopy results.	**4.3. Examining the thin film to confirm species and mixed infections** 1. To confirm the parasite species or mixed infections after examining the thick film, examine the thin film. 2. Place a drop of immersion oil on the feathered edge of the thin film. 3. Move from the 10x lens to the 100x oil immersion lens. 4. Examine the feathery end or edge of the thin film where the red cells lay side by side

Annex 5: Standard Operating Procedures (SOPs) for RDTs

Standard Operating Procedures (SOPs) for RDTs

Materials Required to Perform RDTs

- RDT kit (test cassette, buffer, blood collecting device),
- Sterile lancet,
- Alcohol swab,
- Pencil or pen for labeling,
- Gloves,
- Sharps container,
- Waste disposal container,
- Timer or clock,
- Instruction manual for the specific RDT,
- Dry cotton wool.

PREPARING TO PERFORM THE TEST

1. Gather the necessary materials in the testing area,

2. Check the expiry date at the back of the test package. If the test kit has expired use another test,

3. Ensure the RDT packaging is not damaged by squeezing gently and feel/listen for air leakage.

   Note: if the foil packaging is damaged, use another test kit,

4. Explain to the patient what the test is for and procedure,

5. Open the package tearing along the nick and look for the following

   a. color of desiccant (to be consistent with what indicated by the manufacturer), b. cassette, c. dropper,

6. Remove the cassette from the foil packaging and label it with patient particulars and reading time,

7. Wear a new pair of gloves,

8. Disinfect the puncture site (4^{th} finger of the non-dominant hand) with an alcohol swab or appropriate disinfectant. The 4^{th} finger is preferred because it's the least used and will cause least inconvenience even if it becomes sore.

RDTs PROCEDURE

1. Make a gentle prick towards the pulp (ball) of the 4^{th} finger with a sterile lancet at the disinfected site. Pricking at the tip or midline is more painful. Discard the used lancet in an appropriate sharps' container immediately after use. By applying gentle pressure to the finger express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure no strands of cotton remain on the finger to contaminate blood. Apply gentle pressure to the finger until a new blood drop appears. Emphasize the need for the right skills to ensure correct test performance and accurate results. The reason for wiping out the first drop is because it contains too much tissue fluid which might dilute the antigens and it might be contaminated with the alcohol used for wiping the finger.

2. Using the blood collection device (pipette, inverted cup or capillary tube) provided in the RDT kit, gently immerse the open end in the blood drop. Collect the required volume of blood as per manufacturer 's instructions. Good blood collection and adequate amount of blood are fundamental to ensure good results. After pricking and collecting blood, apply a dry cotton wool at the puncture site to stop the bleeding. Discard the blood collection device in the box for infectious waste.

3. Transfer the collected blood to the sample well (as indicated on the RDT cassette by the manufacturer). It's important to put the sample in the right well as indicated by the manufacturer. Different manufacturers may have different labeling for the different wells. Discard the blood collection device in the box for infectious waste.

4. Holding the buffer bottle vertically, add the recommended number of drops of buffer into the buffer well. Put the exact amount of buffer as indicated by the manufacturer at the correct well of the test device and don't use any other buffer apart from the one provided and specified. Some test kits will come with a bottle of buffer for many tests and others will have enough buffer packed for a single test.

5. Time the test as recommended by the manufacturer. View the result window of the cassette for color band(s).

a. **Negative** – The presence of only a control band, indicates a negative result for *P. falciparum* malaria. If RDT result is negative, alternative causes of fever should be investigated and treated appropriately. Note: Do not read the results before or after the set time. Don't treat any fever as malaria despite a negative result.

b. **Positive** – The presence of both a control band and a test band indicates a positive result.

Refer to manufacturer's instructions to read positive results.

c. **Invalid** — If the test does not show the control band, even if there is test band, the test is invalid. Perform another RDT.

d. Refer to the “RDT Provider job-aid” for pictures of negative, positive and invalid results.

6. Report the results as “RDT Negative” or “RDT Positive” or “RDT Invalid” (in last case the RDT should be repeated. Record patient's information and RDT result in the appropriate register.'

7. Discard the cotton wool, RDT cassette and gloves into the box for infectious waste.
Discard empty bottles/ampulla of buffer, instructions and RDT packaging into the box for non-infectious waste.