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Therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Asayita Primary Hospital, Northeast Ethiopia

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A thesis submitted to the Department of Microbiology, Immunology and Parasitology, School Of Biomedicine And Laboratory Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of a Master of Science Degree in Medical Parasitology.

May, 2025

Addis Ababa, Ethiopia

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May, 2025

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ACKNOWLEDGEMENT

My deep appreciation goes to my advisor, Dr. Tadesse Kebede, for his invaluable support and assistance throughout this project. I am also grateful to my co-advisors, Daniel Getacher (MSc, PhD candidate), Dr. Fitsum Girma, and Dr. Migbaru Keffale, for their encouragement and extensive support during my research journey.

I extend my deepest gratitude to Addis Ababa University, the College of Health Sciences, School of Medicine, Department of Microbiology, Immunology, and Parasitology, and Armaur Hansen Research Institute for their technical and financial support in facilitating this study. I would like to acknowledge All Africa Leprosy, Tuberculosis, and Rehabilitation Training Center Hospital, and Asayita Primary Hospital for their willingness to collaborate on this research. I am especially thankful to the laboratory and data teams at AHRI, as well as ALERT laboratory staff members, including Dr. Tsehaynesh Lema, Belay Merkeb, and Worknesh Debebe for their invaluable guidance throughout the research process. Additionally, I would like to thank the Asayita Primary Hospital study team and participants.

Finally, I would like to express my heartfelt appreciation to my wife, Tsehay Teshome, and my friends for their unwavering support during this journey.

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ABBREVIATIONS AND ACRONYMS

ACPR	Adequate Clinical and Parasitological Response
ACT	Artemisinin-Based Combination Therapy
AHRI	Armauer Hansen Research Institute
AL	Artemether-Lumefantrine
ALERT	All Africa Leprosy, Tuberculosis and Rehabilitation Training Center
AS-AQ	Artesunate-Amodiaquine
CRF	Case Reporting Forms
DBS	Dried Blood Spot
DNA	Deoxyribonucleic Acid
DP	Dihydroartemisinin-Piperaquine
EDTA	Ethylenediaminetetraacitic Acid
ETF	Early Treatment Failure
LCF	Late Clinical Failure
LPF	Late Parasitological Failure
PCR	Polymerase Chain Reaction
PP	Per Protocol
RDT	Rapid Diagnostic Test
SP	Sulphadoxine –Pyrimethamine
SSA	Sub-Saharan Africa
WBC	White Blood Cell
WHO	World Health Organization

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ABSTRACT

Malaria continues to be a significant global health problem, exhibiting various clinical manifestations and differing epidemiological patterns. Since 2004, Artemether-lumefantrine has served as the first line treatment for uncomplicated *P. falciparum* malaria in Ethiopia. Mutations leading to resistance to Artemether-lumefantrine have been identified in several African countries, including Ethiopia, highlighting the critical need for ongoing monitoring of antimalarial drug resistance.Therefore, this study was designed to evaluate the effectiveness of Artemether-lumefantrine to treat uncomplicated *P. falciparum* in the Asayita primary Hospital, Northeast Ethiopia, from December 2023 to March 2024. A single-arm prospective study was carried out to assess the effectiveness of partially observed treatment with Artemether-lumefantrine for uncomplicated *P. falciparum* malaria. The study included 91 participants according to the World Health Organization guidelines, and the participants were partially monitored for 28 days, with regular follow-up visits at Asayita Primary Hospital that included clinical and laboratory (thick and thin blood smears) evaluations. Clinical and sociodemographic data were collected along with whole blood samples from participants. Polymerase chain reaction genotyping of the msp-1, msp-2, and pfpk2 genes was performed for recurrent cases to distinguish between recrudescence and reinfection. The data was collected using standard case report forms. Based on the evaluations, participants were classified as having early or late threatment failure or adequate response. The effectiveness of the drug was measured by the percentage of participants achieving adequate clinical and parasitological response. After cleaning and validating the data, analysis was conducted using stata 14 and RStudio. Among the 91 participants enrolled, 73 completed the 28-day follow-up period. Artemether-lumefantrine achieved a 100% fever clearance by day 2 and a 95.4% clearance rate of asexual parasites by day 3. Two cases of recurrent malaria were confirmed as reinfections by PCR genotyping. Per protocol analysis showed cumulative success rates of 97.3% (PCR-uncorrected, 95% CI: 89.7–99.4) and 100% (PCR-corrected). These findings show Artemether-lumefantrine's high effectiveness and tolerability in treating uncomplicated *P. falciparum* malaria, while underscoring the need for continuous monitoring to address potential artemisinin resistance.

Keyword: Therapeutic effectiveness, Artemether-lumefantrine, Malaria, Ethiopia

1. INTRODUCTION

1.1. Background

Malaria remained a significant global health challenge in 2023, with 263 million cases and 597,000 deaths reported worldwide (WHO, 2024b). The approximately 11 million cases increase from 2022 was largely attributed to the WHO African Region, accounting for 89.7% of the rise, and the WHO Eastern Mediterranean Region, contributing 15.5 (WHO, 2024b). The main countries responsible for the rise in malaria cases include Ethiopia, Madagascar, Pakistan, the Democratic Republic of Congo, and Nigeria. The malaria incidence rate has increased to 58.4 cases per 1,000 individuals at risk. However, there was a consistent decline in malaria-related deaths, decreasing from 864,000 in 2000 to 576,000 in 2019 (WHO, 2023b). However, in 2020, there was an increase of 55,000 malaria deaths due to disruption in access to prevention and treatment tools caused by the coronavirus disease 2019 (COVID-19) pandemic (Rivas-Morello *et al.*, 2023, WHO, 2023b). The malaria mortality rate decreased by half between 2000 and 2019, going from 28.8 deaths per 100,000 at-risk populations to 14.1, however, in 2020, the mortality rate increased to 15.2 before slightly declining to 14.5 in 2021 and 14.3 in 2022 (WHO, 2023b).

Among children under five, the percentage of malaria-related mortality dropped from 86.8% in 2000 to 76.0% in 2022 (WHO, 2023b). Ethiopia is predicted to further reduce its malaria burden in 2020 from the 2016 baseline by 40% and eliminate the disease from the country by 2030 (FMOH, 2021). Malaria is the leading infectious disease in Ethiopia, affecting over 68% of the population. The most common species responsible for malaria cases are *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*), accounting for approximately 60% and 40% of infections, respectively. *Plasmodium falciparum* is particularly responsible for causing severe malaria, as it leads to end-organ damage through the adherence of infected red blood cells (RBCs) to the vascular endothelium. Severe malaria predominantly occur from *P. falciparum* and has a high mortality rate, reaching up to 10% in adults and 33% in children under the age of 12 (Assefa *et al.*, 2010, Plewes *et al.*, 2018).

Malaria in humans is caused by five species of the *Plasmodium* parasite: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. Malaria transmission takes place principally via the bite of an infected female Anopheles mosquito. However, congenital and blood transmission may also contribute to transmission (Sato, 2021). The infective stage, called sporozoites, enters the liver

cells and forms schizonts containing merozoites. The released merozoites enter red blood cells, multiply, and lead to the pathophysiology and the onset of malaria symptoms (Bartoloni and Zammarchi, 2012).

The most common indication of malaria is a fever or a recent history of fever, but given that many other illnesses likewise present with fever in areas where malaria is common. An inaccurate diagnosis can result in an improper use of antimalarial medications, while undetected cases lead to disease complications, death, and antimalarial drug resistance. Various methods, from microscopy to molecular techniques, are available for detecting malaria parasites (Fitri *et al.*, 2022). While clinical diagnosis based on symptoms and physical examination is still used in some areas (Wogu and Nduka, 2018). Rapid diagnostic tests (RDTs) are widely used to identify specific antigens or antibodies linked to the targeted pathogen (Mihreteab *et al.*, 2023, WHO, 2023b). While molecular techniques like polymerase chain reaction (PCR) deliver greater sensitivity and specificity, they are less practical in resource-constrained settings due to the need for advanced equipment and trained personnel (Fitri *et al.*, 2022, Tedla, 2019).

Severe malaria complications primarily affect non-immune individuals. These complications involve various body systems, including the central nervous, pulmonary, renal, and hematopoietic systems. Prompt assessment and treatment are crucial as severe malaria can be rapidly fatal. Cerebral malaria can lead to coma (Bartoloni and Zammarchi, 2012). In uncomplicated *P. falciparum* malaria, fever occurs a few days after initial symptoms and within 9-14 days of the incubation period. Other symptoms worsen during the prodromal phase. Physical findings include hepatosplenomegaly, jaundice, rapid pulse, and low blood pressure. Laboratory tests may show thrombocytopenia and elevated liver enzymes.

Early treatment is necessary to prevent progression to severe malaria, but with appropriate treatment, the prognosis is generally good. Treatment typically resolves fever and symptoms within three days, but recrudescence may occur (Bartoloni and Zammarchi, 2012). Malaria, caused by *P. falciparum*, is a major challenge in Africa, where most cases and deaths occur. The World Health Organization recommends the use of Artemisinin-based combination therapies (ACT) as the primary treatment for uncomplicated *P. falciparum malaria*. This approach involves a fast-acting artemisinin drug to rapidly decrease the parasite load in the body, combined with a slower-acting partner drug to ensure the elimination of any remaining parasites.(Sa *et al.*, 2018).

In the early 1990s, Ethiopia faced a major threat to malaria prevention and control with the emergence of a chloroquine-resistant *P. falciparum* strain. Consequently, in 1998, the country adopted sulphadoxine-pyrimethamine (SP) as the Primary treatment option. In 2003, a study identified a significant failure rate in SP treatment, which resulted in a widespread malaria epidemic. As a response, Ethiopia adopted Artemether-lumefantrine (AL) as the first line treatment for *P. falciparum* malaria in 2004. Artemether-lumefantrine has a high cure rate and a low treatment failure rate, making it an effective option (Getnet *et al.*, 2015, Gubae *et al.*, 2023). While it has been effective in treating the parasite, it is now important to also focus on reducing transmission of the disease. Interrupting the transmission cycle between mosquitoes and humans is crucial for the success of malaria elimination strategies (Karunajeewa and Mueller, 2016).

Artemisinin-based combination therapy, along with the larval source management, use of insecticide-treated bed nets, and indoor residual spraying, has been effective in managing malaria in African countries, including Ethiopia (FMOH, 2018). However, adverse events such as abdominal pain, nausea, and vomiting have been observed during treatment (Olivera *et al.*, 2020). While ACT has shown high cure rates, there have been reports of emerging drug resistance in some regions. Therefore, ongoing monitoring, such as regular therapeutic efficacy studies, are necessary to ensure the continued effectiveness of ACT in Ethiopia (Gubae *et al.*, 2023, WHO, 2022). This study focused on assessing the effectiveness of AL as a treatment for uncomplicated *P. falciparum* malaria in the Asayita primery Hospital, northeastern Ethiopia.

1.2. Statement of the Problem

Malaria parasites in humans remain a significant public health issue in Sub-Saharan Africa (SSA) leading to a high number of cases and deaths worldwide. In 2023, there has been a further increase in cases related to 2022. The WHO African Region recorded 94% of cases and 95% of deaths globally in 2023; Children under five accounted for 76% of all deaths in this region(WHO, 2024b). In the past two years, Ethiopia has faced several challenges, including ineffective malaria prevention and control in conflict zones, the rise of the new malaria vector *Anophiles stephensi*, insecticide resistance in vectors, and the impacts of climate change and variability (WHO, 2024a). According to the WHO malaria report, from 1 January to October 20, 2024, Ethiopia reported 7.3 million malaria cases and 1,157 deaths (WHO, 2024b).

Artemether-lumefantrine is the currently recommended first-line treatment for uncomplicated malaria caused by *P. falciparum* and Dihydroartemisinin–piperaquine (DP) is used for severe cases (EMOH, 2022). However, resistance to antimalarial drugs has become a recurring issue in malaria control efforts (Li *et al.,* 2022). The misuse and self-treatment of antimalarial drugs further exacerbate the problem of drug resistance. Some individuals choose to purchase antimalarial medications from private stores without a proper diagnosis or instructions. Therefore it is crucial to ensure that antimalarial medications are used correctly and responsibly to preserve their efficacy (Zhou *et al.,* 2020). Artemisinin-based drugs have been very successful in treating uncomplicated cases of malaria worldwide. However, there are new strains of *P. falciparum* that have become resistant to artemisinin in parts of Southeast Asia (St. Laurent *et al.*, 2015), making its effectiveness questionable. The potential spread of these resistant strains to Africa, where most malaria-related deaths happen, could lead to devastating outcome (Ayalew, 2017, Bayih *et al.*, 2016).

The key component in fighting against malaria is effective treatment, but the emergence of resistance to artemisinin and its partner drugs poses a significant threat. Artemisinin partial resistance has been detected in four African countries, which is of great concern and requires an urgent response (Uwimana *et al.*, 2021). The effectiveness of antimalarial drugs is monitored through a therapeutic efficacy study which assesses treatment outcomes in patients. Polymerase chain reaction correction is necessary to accurately determine if treatment failure is due to reinfection or recrudescence. Therapeutic efficacy studies are considered the gold standard for determining national treatment policies (WHO, 2023b). In the WHO African Region, the

recommended first-line therapies for treating *P. falciparum* malaria consist of AL, AS-AQ, Artesunate-Pyrimethamine, and DP (Ogutu, 2013). Most studies showed good efficacy of those drug. However, failure rates of greater than 10% following treatment with AL, as recommended by the WHO PCR correction methodology, have been reported in specific studies in the WHO African Region between 2015 and 2022 (Dimbu *et al.*, 2021, WHO, 2023b). Research conducted in Africa has shown that artemisinin is less effective, with slower clearing periods and higher rates of recrudescence (Plucinski *et al.*, 2017). There have been reports of kelch13 (K13) gene mutations linked to partial resistance to Artemisinins in Tanzania, Rwanda, and Uganda (Conrad *et al.*, 2023, Owoloye *et al.*, 2021, Uwimana *et al.*, 2020).

Molecular surveillance has detected Artemisinin resistance in Eritrea, Rwanda, Uganda, and the United Republic of Tanzania associated with specific mutations in the PfKelch13 gene (Conrad *et al.*, 2023, Mihreteab *et al.*, 2023, Owoloye *et al.*, 2021, Uwimana *et al.*, 2020). The R622I mutation and Pfhrp2/3 deletions have been found in Eritrea and Ethiopia (Alemayehu *et al.*, 2021, Mihreteab *et al.*, 2023). The prevalence of the Pfkelch13 R622I mutation in Eritrea has significantly increased from 8.6% in 2016 to 21.0% in 2019 (Mihreteab *et al.*, 2023). In Ethiopia, the highest prevalence of the R622I mutation was in Amhara (9.8%), followed by Tigray (8.4%), and Gambella (3.6%) (Fola *et al.*, 2023). The United Republic of Tanzania has become the fourth African nation to identify artemisinin resistance, demonstrating delayed parasite clearance following treatment with AL and AS-AQ, as well as a significant prevalence of the R561H mutation (Mihreteab *et al.*, 2023, WHO, 2023b). In Ethiopia, where malaria is prevalent and people are still suffering from it there is a need to monitor and continually collect data on the effectiveness of first-line ACT. Currently, the rise and spread of drug resistance pose a significant threat to the effectiveness of ACT.

1.3. Significance of the Study

The outcomes of this study will help to: -

- ✓ Providing updated evidence on the therapeutic effectiveness of AL in treating uncomplicated *P. falciparum* malaria
- ✓ Support early detection of emerging antimalarialdrug resistance
- ✓ Improves patients care by ensuring the continued use of effective first-line therapies

2. LITERATURE REVIEW

2.1. Artemisinin-Based Combination Therapy

In recent years, ACTs has increasingly been administered specifically to patients who test positive for malaria, due to an increase in diagnostic tests. The introduction of ACTs in Africa was prompted by clinical failures and the emergence of resistance to both SP and chloroquine (Roux *et al.*, 2021). World Health Organization recommends ACTs, which are currently widely regarded as the best choice for treating uncomplicated *P. falciparum* malaria. Artemisinin-based combination therapy consists of an artemisinin derivative combined with another antimalarial drug that is structurally different and more slowly removed antimalarial drug from the body (WHO, 2023b). They work quickly and consistently. For AS-AQ, AL, and DP, the drug's effectiveness is assessed by pairing it with the artemisinin derivative; this usually exceeds 95% (Nosten and White, 2007). The cost, effectiveness, safety, rate of reinfection, and other considerations all play a role in selecting the right ACT (Derbie *et al.*, 2020).

2.1.1. Artemether-Lumefantrine (Coartem®) as Treatment

In response to reports of resistance to partner medicines, a co-formulated tablet containing AL was introduced for treating uncomplicated *P. falciparum* malaria. Specifically, a commercially available fixed-dose combination includes 20mg of artemether and 120mg of lumefantrine (Krishna *et al.*, 2021). Currently, AL has been authorized in 86 countries, with 30 of the 47 SSA nations employing it as their first-line therapy and 8 using it as their second-line treatment (Ogutu, 2013).

Artemether-lumefantrine are blood schizonticides with different mechanisms of action and compatible pharmacokinetics, they have synergistic anti-malarial effects. Derivatives of artemisinin are active against all human-infecting *Plasmodium* species and exhibits a faster onset of anti-parasitic activity compared to all other anti-malarial drug currently on the market (Ogutu, 2013). Crucially, they permit greater clearance of parasites than any other anti-malarial medication (parasite counts can be lowered by 10^4 every asexual cycle) (White *et al.*, 1999). Shorter treatment courses (three days) become beneficial when paired with an effective anti-malarial drug with a slower clearance rate, like lumefantrine. While the majority of circulating malaria parasites are killed by artemether and its active metabolite, dihydroartemisinin, which has a half-life of about two hours, leading to a quick resolution of symptoms. In contrast, lumefantrine is metabolized into

desbutyllumefantrine, which possesses a half-life of 3 to 6 days and provides a high long-term cure rate following a brief treatment period (White *et al.*, 1999). Consequently, the combination quickly eliminates parasitemia and the majority of symptoms associated with malaria while also preventing recrudescence market (Ogutu, 2013).

2.1.2. Anti-Malarial Drug Resistance

Resistance of *P. falciparum* to commonly used antimalarial medications like chloroquine and SP was the major obstacle to controlling malaria in areas where the disease is endemic, particularly in SSA (Derbie *et al.*, 2020). Drug resistance to antimalarials has been described as "the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the tolerance of the subject". This definition was later adapted to specify that the drug in question must "gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action" (WHO, 2020).

Factors such as prolonged use of artemisinin, monotherapies like amodiaquine, mefloquine, and SP, and incomplete treatment in semi-immune patients can promote malaria drug resistance. Previously, treatment strategies varied based on immunity status, but this is no longer recommended due to variability in individual immunity. Ensuring full ACT courses once malaria is confirmed and avoiding partial treatment are crucial to prevent resistance and ensure effective care(WHO, 2023a).

2.2. Studies on the Therapeutic Effectiveness of Artemether Lumefantrine

A study in Colombia found that AL was highly effective in treating uncomplicated malaria caused by *P. falciparum* in Choco, Colombia. The results showed that almost all participants (98.8% without PCR correction and 100% with PCR correction) were cured of their infection within 28 days. Out of 88 patients treated, 84 (98.8%) showed a good response to the treatment by day 28, and only one patient had a small amount of parasites remaining, but these did not grow during further testing. Additionally, the study found no evidence of resistance to artemisinin due to genetic mutations in the *P. falciparum* gene (Olivera *et al.*, 2020).

A study in Nepal examined the effectiveness of ACT for treating malaria. Among 56,013 suspected cases, 120 were confirmed to have *P. falciparum* malaria. Out of these, 24 participants enrolled in the study and successfully completed a 28-day follow-up period. One patient defaulted from treatment, but the remaining 23 patients showed a response to the treatment. The study also checked for the K13 mutation, a marker for ACT resistance. Fortunately, no mutation was detected on day 1, indicating that ACT resistance had not yet developed at the molecular level (Ghimire *et al.*, 2018).

A study was conducted in four malarious regions of India to assess the effectiveness of AL in treating uncomplicated *P. falciparum* malaria. The results showed that almost all participants (98% without PCR correction and 99% with PCR correction) were cured of their infection within 28 days. The cure rate was 100% at three of the study sites, but slightly lower (92.5%) at one site. No participants experienced early treatment failure, and PCR testing revealed that one patient's infection recurred due to recrudescence, while two patients experienced late parasitemia due to recrudescence. The average duration for the fever to resolve was 27.2 hours, while the average time for the parasites to be cleared was 30.1 hours. No adverse effects were reported during the study (Bharti *et al.*, 2016).

A study was conducted to compare the effectiveness of two malaria treatments, AS-AQ and AL, in Liberia. The study involved 359 children, with 180 receiving AS-AQ and 179 receiving AL. Both treatments showed high effectiveness, with 90-93% of patients not having parasites after treatment with AS-AQ and 100% with AL, the study also found that two patients had parasites after three days, and no signs of artemisinin resistance were detected. (Koko *et al.*, 2022).

Two studies conducted in Angola assessed how children with uncomplicated P. falciparum infections responded to various treatments, while also examining molecular resistance markers in cases of treatment failure. In the first study, 467 children were treated with AL, AS-AQ, or DP, and 54 treatment failures were observed. The efficacy of AL ranged from 88.1% to 96.3. All treatment failures exhibited wild-type K13, but the AL failures had pfmdr1 haplotypes linked to reduced susceptibility to lumefantrine (Plucinski *et al.*, 2017). In the second study, children in Benguela, Lunda Sul, and Zaire were treated with AL or AS-AQ, and genetic analysis was conducted on treatment failure samples. Clearance rates on day 3, all groups had clearance rates of 95% or higher. By day 28, the uncorrected effectiveness estimates ranged from 84.2 to 90.1%

for AL and 84.7 to 100% for AS-AQ. After adjusting for molecular corrections, the estimates increased to 87.6 to 98.4% for AL and 95.6 to 100% for AS-AQ (Dimbu et al., 2021).

A study was conducted in the Democratic Republic of the Congo to evaluate the effectiveness of three malaria treatments, AL, AS-AQ, and DP, in treating uncomplicated malaria in children aged 6 to 59 months. The study monitored the treatments' efficacy at 28 days for AL and AS-AQ and 42 days for DP, both with and without PCR correction. The results showed that the uncorrected efficacy of AL and AS-AQ varied across different locations, ranging from 63% to 88%, while the PCR-corrected efficacy ranged from 86% to 98% (Moriarty *et al.*, 2021).

A study in Mozambique tested the effectiveness and safety of two malaria treatments, AL and AS-AQ, in children aged 6 to 59 months. The results showed that both treatments were highly effective in treating uncomplicated *P. falciparum* malaria, with AS-AQ having a slightly higher efficacy rate than AL. Additionally, most recurrences of malaria were due to new infections, rather than treatment failure. Both treatments had minimal adverse effects, with less than 2% of participants experiencing side effects (Nhama *et al.*, 2021).

A single-arm prospective observational study was carried out at Teda Health Centre in Northwest Ethiopia from September 2022 to February 2023, aiming to evaluate the cure rate of AL for uncomplicated *P. falciparum* malaria following the administration of a single low dose of primaquine. The study revealed that the Kaplan-Meier success rates on day 28 were 95.8% before PCR uncorrected and 97.3% after PCR corrected. Per-protocol analysis showed adequate clinical and parasitological response rates of 95.5% PCR-uncorrected and 97% PCR-corrected. By day 3, 97% of the participants had cleared asexual parasitemia, and all participants were free of fever by day 2. Additionally, all patients with gametocytes at baseline tested negative for them by day 2. Hemoglobin levels showed an average increase from 13.10 g/dl on day 0 to 13.27 g/dl on day 14, and further increased to 13.69 g/dl on day 28, according to a paired sample t-test. The reported adverse events were mild in nature.(Woldesenbet *et al.*, 2024)

In 2023, a study at the Shecha Health Centre in Arba Minch, southern Ethiopia, found that the PCR-corrected cure rate for the treatment being evaluated was 98.6%, with no presence of asexual parasites by day 2. Additionally, the study indicated that there were no serious adverse events

noted during the 28-day follow-up. The most frequently reported mild adverse events included headache, nausea, loss of appetite, and vomiting (Gubae *et al.*, 2023).

A recent study conducted in Chewaka District, Ethiopia, in 2020, reported an impressive PCR-corrected cure rate of 96% among 80 participants. Particularly, only three individuals tested positive for the malaria parasite on day three, demonstrating the rapid clearance associated with the AL regimen. Furthermore, the study noted that there were no significant adverse events throughout the 28-day follow-up period. While 20% of participants experienced mild adverse events such as headache, vomiting, shortness of breath, cough, diarrhoea, and joint pain, most of these possible adverse reactions subsided as the parasitemia resolved. The cough was an exception, lingering for some time after the parasites had cleared (Abamecha *et al.*, 2020).

A study was conducted at Kola Diba Health Center to evaluate the effectiveness and safety of AL in treating uncomplicated *P. falciparum* mono-infection. Patients were treated with AL for three days and then monitored for 28 days following the WHO protocol. A total of 80 participants were enrolled in the study, and 75 completed the 28-day follow-up pariod. There were no significant adverse events reported during the study, and treatment with AL eliminated parasitemia and fever in 95% of participants by day 3. However, the study also noted 6 cases of LPF, which make up 8% of the total participants. The uncorrected per protocol cure rate of AL was determined to be 92%, with a 95% confidence interval ranging from 85.7% to 98.3% (Deressa *et al.*, 2017).

A single-arm study was conducted from July to October 2022 in the Arba Minch Zuria District of the Gamo Zone in Southwest Ethiopia to assess the effectiveness of AL for the treatment of uncomplicated *P. falciparum* malaria. A total of 69 patients participated in the study, with 67 successfully completing the 28-day follow-up. By day 2, AL achieved a 100% fever clearance rate, and by day 3, all asexual parasites were eliminated. Five individuals experienced recurrent malaria; two of them were reinfected, and three of them experienced a relapse. The cumulative incidence of success, both PCR-uncorrected and PCR-corrected, was 93.7% (95% CI 85.5–97.3) and 96.2% (95% CI 85.5–98.7) respectively (Daka *et al.*, 2024).

In a study of Artemether-lumefantrine treatment for patients with *P.falciparum* malaria aged 5 or older in Bahir Dar district from March to July 2012, nearly all participants (96.1%) were cleared of parasites by day 3 and almost all (98.5%) showed a complete response to the treatment by the

end of the study. Only a small number of patients (1.5%) experienced late treatment failure between days 7-14, and only a minor (1.3%) still had anaemia at the end of the study (Ebstie *et al.*, 2015).

Artemether-lumefantrine, are highly effective and safe options for treating *uncomplicated P. falciparum* malaria across different regions, with cure rates typically exceeding 90% within 28 days. Rapid parasite clearance, minimal adverse effects, and the absence of significant resistance markers such as K13 mutations highlight the current robustness of these treatments. While occasional treatment failures due to recrudescence or reinfection have been reported, they are generally not linked to resistance. These consistent results underscore the importance of continued monitoring and molecular surveillance to sustain the efficacy of ACT regimens and support ongoing malaria control efforts worldwide.

3. OBJECTIVES

3.1. General Objective

- To evaluate the therapeutic effectiveness of AL for the treatment of uncomplicated *P. falciparum* in the Asayita Primary Hospital in Northeast Ethiopia from December 2023 to March 2024

3.2. Specific Objectives

- To assess the clinical and parasitological cure rate of AL in patients with uncomplicated *P. falciparum* malaria
- To differentiate between recrudescent and reinfection among recurrent *P. falciparum*cases using molecular genotyping
- To evaluate the adverse drug reactions (ADRs) associated with the administration of AL for *P. falciparum* treatment

4. MATERIALS AND METHODS

4.1. Study Area

Asayita district, located in Zone One of the Afar Regional State, lies 652 km northeast of Addis Ababa Based on projections from the 2013 National Census conducted by the Central Statistical Agency of Ethiopia, the total population of the woreda was 66,780, comprising 35,572 males and 31,208 females. Geographically, it spans from 11° 08' 27" to 11° 40' 40" N latitude and 41° 25' 15" to 41° 52' 30" E longitude, with elevations ranging between 350 and 473 meters above sea level. The area experiences an annual mean temperature exceeding 27.5°C, and its yearly rainfall falls between 500 mm and 1000 mm. In this district, 8% of suspected malaria cases were confirmed through microscopy, with 94.2% attributed to *P. falciparum* and 5.8% (222 cases) to *P. vivax*(Belay, 2018).

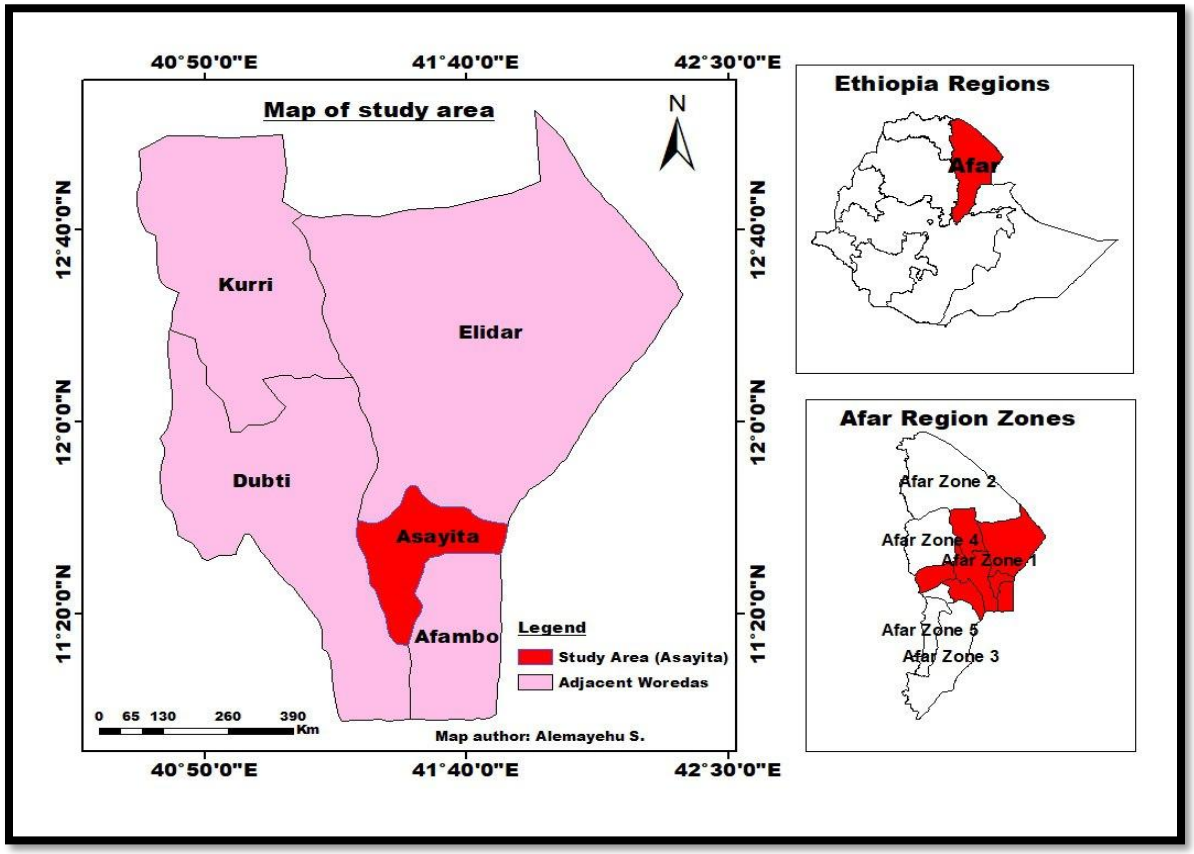

Figure 1: Locational map of the study area

4.2. Study Design

A single-arm prospective study was condacted to evaluation the effectiveness of AL the treatment of uncomplicated *P. falciparum* malaria based on the who revised protocol during malaria transmission season from December 2023 to March 2024(WHO, 2009).

4.3. Study Population

The population of interest involved patients 6 months of age and older who lived within a 20 km radius of the Hospital catchment area and were diagnosed with uncomplicated *P. falciparum* mono-infection.

4.4. Study Period

The study carried out during the malaria transmission season, from December 2023 to March 2024. Participants were observed over a 28-day period, with follow-ups scheduled on days 0, 1, 2, 3, 7, 14, 21, and 28.

4.5. Inclusion and Exclusion Criteria

4.5.1. Inclusion Criteria

The participant must be living within 20 km of the catchment of the health facility with a traceable address/phone number, volunteers to give their address/phone number, be willing not to travel for the duration of the study, comply with the study protocol and visit schedule, and give informed consent, assent, or parental/guardian permission. Age ≥ 6 months, axillary temperature ≥ 37.5º C or history of fever during the previous 24 hours and weight ≥ 5.0 kg. Additionaly, participant must have a mono-infection with *P. falciparum.*

4.5.2. Exclusion Criteria

The exclusion criteria of this study include, individuals who are unable to swallow oral medications, or who exhibit general danger signs or severe symptoms associated with malaria, will not be eligible. Additionally, those individuals diagnosed with severe anemia (hemoglobin levels below 7 g/dl), and they include significant or chronic health conditions, such as heart, kidney, or liver diseases, as well as sickle cell disease and HIV/AIDS, and women who are pregnant or breastfeeding are also excluded. Women of childbearing age (defined as ages 12–49 or menstruating) who refuse to undergo pregnancy tests were also excluded. Furthermore, individuals

with a history of hypersensitivity to the study or rescue medications, those taking regular medications that may affect the pharmacokinetics or efficacy of antimalarial treatments. Finally, children aged 6-59 months exhibiting severe malnutrition, characterized by symmetrical edema including at least the feet or a mid-upper arm circumference of less than 110 mm, will also be excluded from the study.

4.6. Sample Size Determination

The sample size was determined in accordance with the WHO 2009 guidelines, using the single population proportion formula. This calculation factored in a 5% margin of error, a 95% confidence level, a 5% treatment failure rate, and a projected dropout rate of 20%. A minimum sample size of 88 was calculated, and a total of 91 patients were enrolled in the study, exceeding the calculated minimum requirement (WHO, 2009).

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

$= (1.96/0.05)^2\ 0.05\ (1\text{-}0.05) = 73$; $n = (1 + 0.2)73 = 88$

Where n = a total sample size used, Zα/2 = 1.96 which corresponds to 95% confidence interval (CI), d= the margin of the error (5%), p = the expected treatment failure (5%), Participants were selected from symptomatic patients suspected of having malaria who visited the Hospital. Adults and children over 6 months of age, who satisfied the inclusion criteria and provided informed consent, were eligible to take part in the study.

4.7. Sampling Technique

A convenient sampling technique was employed to recruit outpatient participants until the desired sample size was reached. Study nurses conducted patient screenings in the outpatient department to identify individuals who met the study's inclusion criteria. Additionally, the health professionals responsible for data collection received training on the procedures outlined by the WHO (WHO, 2009).

4.8. Study Variables

4.8.1. Dependent Variable

- Therapeutic efficacy of AL

4.8.2. Independent Variable

- Patient demographics (age, gender)
- Parasite density at baseline
- Adherence to the prescribed treatment regimen
- Nutritional status

4.9. Operational Definitions

Uncomplicated malaria: A patient who shows symptoms consistent with malaria and has a positive test result (either through microscopy or RDT) but does not exhibit any signs of severe malaria.(WHO, 2015).

4.10. Classification of Treatment Outcomes

Early Treatment Failure (ETF):

- Presence of parasitemia alongside danger signs or severe malaria on day 1, 2, or 3.
- Parasitemia levels on day 2 exceed those recorded on day 0, regardless of axillary temperature.
- Parasitemia on day 3 is accompanied by an axillary temperature of 37.5 °C or higher.
- Parasitemia on day 3 constitutes 25% or more of the count observed on day 0.

Late Clinical Failure (LCF):

- Danger signs or severe malaria occurring alongside parasitemia on any day between day 4 and day 28 in patients who did not previously meet the criteria for early treatment failure.
- Presence of parasitemia on any day between day 4 and day 28, combined with an axillary temperature of 37.5 °C or higher, in patients who did not previously meet the criteria for early treatment failure.

Late Parasitological Failure (LPF):

- Parasitemia present on any day from day 7 to day 28 (or day 42), with an axillary temperature below 37.5 °C, in patients who did not previously meet the criteria for early treatment failure or late clinical failure.

Adequate Clinical and Parasitological Response (ACPR)

- Absence of parasitemia on day 28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure, or late parasitological failure (WHO, 2009).

4.11. Clinical Evaluation

Clinical evaluations were conducted at the time of enrollment, during scheduled visits on Days 0, 1, 2, 3, 7, 14, 21, and 28, and additionally during any unscheduled visits. At baseline, a comprehensive physical examination was performed, which included recording a detailed medical history, body temperature, demographic data, and contact details. Body weight and temperature were measured at enrollment and during each follow-up visit, with the weighing scales properly calibrated before hand. The screening weight was utilized for determining eligibility criteria and calculating the treatment dosage to be administered. Participants were instructed to remove excessive clothing prior to being weighed, and their weight was documented to the nearest kilogram. A digital thermometer, with a precision of 0.1°C, was employed to measure body temperature, consistently using the same measurement method throughout the study. If the initial temperature reading registered below 36 °C, the measurement was repeated.

4.12. Laboratory Examination

4.12.1. Sample Collection Procedure

Approximately 500 µL of blood was obtained via finger prick and collected in EDTA-coated microtainer tubes supplied by MMS medical and laboratory supplies. The sample was used for various purposes: preparing thick and thin smears for microscopic examination of malaria parasites, assessing hemoglobin levels with a portable spectrophotometer standard G6PD test™ analyzer, and preparing dried blood spots (DBS) comprising five 20 µL spots on filter paper and the DBS samples were individually packaged into a double zip-lock plastic bag with desiccant. The leftover blood sample was retained in the EDTA tubes for the purpose of DNA extraction. The sample that was collected throughout the study period was stored at -20°C to ensure the parasite DNA integrity. These samples were gathered at the time of enrollment and on follow-up days 1, 2, 3, 7, 14, 21, 28, as well as on the day of any treatment failure.

4.12.2. Blood Film Preparation and Microscopic Examination

Parasite screening and quantification procedures began with the collection of thick and thin blood smears on Day 0 during the initial screening. These smears were used to confirm adherence with the study's inclusion and exclusion criteria. Thick blood smears were subsequently examined on follow-up days, including Days 1, 2, 3, 7, 14, 21, and 28, as well as on any unscheduled visit. Microscope slides were labeled using study number, alongside the corresponding follow-up day and collection date.

A freshly prepared 10% Giemsa stain dilution was used to stain both thick and thin blood films, which were then examined under a microscope at 1000× magnification to identify the parasite species and assess parasite density. To determine parasite density, the count of asexual parasites was performed across 200 white blood cells (WBC) using a hand tally counter. If the parasite count exceeded 500 before reaching 200 WBC, the tally was stopped after completing the reading of the last field. When the count of asexual parasites was less than 10 per 200 WBC in follow-up smears, the examination continued until at least 500 WBC had been evaluated. A blood film was considered negative if no asexual parasites were found after examining 1000 WBC. To assess gametocytes density during the enrollment day and on subsequent follow-up pariod, a total of 1000 WBC were counted.

When discrepancies in parasite positivity, species identification, or density exceeded 10%, a third blinded microscopist reviewed the blood slides. For determining species and positivity, the final results were based on the agreements between the two technicians, whereas for parasite density, the final estimate was the average of the two closest density values.

The number of parasites/µl of blood was calculated by using:

$$\text{Asexual parasites density/µl} = \left(\frac{\text{parasites counted}}{\text{200 WBCs counted}}\right) 8000$$

$$\text{Gametocyte density/µl} = \left(\frac{\text{gametecyte counted}}{\text{1000 WBCs counted}}\right) 8000$$

4.12.3. Hemoglobin Measurement

Hemoglobin levels were assessed from capilary blood samples using a portable spectrophotometer, the standard G6PD test™ analyzer produced by SDBIOSENSOR, on days 0, 7, 14, 21, and 28.

4.12.4. Pregnancy Test (if older children/adults are included in the study)

Female of reproductive age who were menstruating or between the ages of 12 and 49 were asked to take a urine pregnancy test manufactured by Life Care Pharmaceuticals trading Addis Ababa with 99% accuracy were used before enrolment to the study because AL is contraindicated during the first trimester. If they had missed their first expected period by a week or more after enrolling in the study, they also took another urine pregnancy test on day 28 or earlier if they withdrew from the study.

4.12.5. DNA Extraction and PCR Amplification

Parasite DNA was extracted from whole blood samples collected during scheduled follow-up and unscheduled visits using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), in according to the manufacturer's instructions. The extracted DNA was subsequently analyzed through quantitative PCR (qPCR) to detect and quantify *P. falciparum* and *P. vivax* parasites. The qPCR was conducted with TaqMan Fast Advanced Master Mix (Applied Biosystems, #4444964), utilizing primer and probe concentrations of 300 nm for primers and 111 nm for the probe targeting *P. falciparum,* as well as 200 nm for primers and 110 nm for the probe targeting *P. vivax*. The assay was designed in a multiplex format allowing for the concurrent detection and quantification of both parasite species within a single reaction. Standard curves were generated through serial dilutions of plasmids, with the *P. falciparum* NF54 plasmid beginning at 10^6 copies/ml and the *P. vivax* 18S plasmid starting at 10^7 copies/ml. These standards were diluted down to 10^2 copies/ml for *P. falciparum* and 10^3 copies/ml for *P. vivax*. These standard curves served as a reference for quantification. Each qPCR reaction was carried out in a final volume of 20 µl, incorporating 5 µl of the extracted DNA as the input. The primers and probes were specifically designed to target the 18S rRNA gene of both *Plasmodium* species.

4.12.6. Molecular Genotyping

Parasite DNA was extracted from whole blood samples on Day 0 and the day of treatment failure using the QIAamp® 96 DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) and was aliquoted for genotyping and sent to the London School of Hygiene and Tropical Medicine for further analysis. For cases classified as LCF or LPF, a nested PCR analysis was performed on paired whole blood samples from Day 0 and the day of treatment failure. Polymerase chain reaction

corrections were applied based on fragment size analysis using capillary electrophoresis. In accordance with WHO informal recommendations for the African region, using microsatellite markers instead of glurp, three molecular markers merozoite surface protein 1 (*msp1*), merozoite surface protein 2 (*msp2*), and *P. falciparum* protein kinase 2 (*PfPK2*) were used for the primary endpoint analysis.

4.13. Treatment and Dosing Procedure

Participants in the study received the standard six-dose regimen of AL, which was administered twice daily for three days (Days 0, 1, and 2) as outlined in Annex 4. Unique identifiers for the study participants were created and assigned according to their first visit date. Treatment with AL commenced after sample collection on Day 0. Eligible individuals who voluntarily agreed to participate were enrolled in the study. The same batch of AL (Batch No HWE1213092, Exp. 05/2026), containing 20 mg of Artemether and 120 mg of Lumefantrine, was sourced from Ipca Laboratories Ltd. in India, and acquired through the national malaria program. This batch, imported for the treatment of malaria patients from a WHO pre-approved supplier, underwent pre-shipment quality testing and was utilized for treating infected individuals throughout the study period.

The study nurse closely monitored the individual's first medication dose at the Hospital, ensuring that they ate a fatty food before taking it for improved absorption. The second dose was given 8 hours after the first dose on Day 0. On Days 1 and 2, participants received their first dose of drug under the supervision of study nurse in the morning, followed by a self-administered evening dose at home, either by the participant or a caregiver. After the supervised dose, participants were monitored for 30 minutes for any adverse reactions or incidents of vomiting. In cases where vomiting occurred during this observation period, participants were administered the same dose again and observed for an additional 30 minutes. If vomiting persisted, the participant would be referred for higher-level medical care, specifically for parenteral artesunate treatment, in accordance with standard anti-malarial protocols (EMOH, 2022), and subsequently withdrawn from the study.

4.13.1. Other Medication

Following established protocols, healthcare providers delivered supportive care to patients as required. Participants with an axillary temperature of ≥38°C at enrollment were given paracetamol at a standard dose of 10 mg/kilogram in the form of tablets or syrup, administered with a 6-hour difference as needed until their next visit. Each enrolled participant received at least two doses of paracetamol that were given for home use. Additionally, patients or their parents/guardians were advised on the proper methods of tepid sponging and fanning. Any patient with treatment failure or recrudescence will receive DP with the recommended dose: for children 5 to < 25 kg: 2.5 to 10 mg/kg daily of DHA plus 20 to 32 mg/kg daily of PPQ, and for children 25 kg and over and adults: 2 to 10 mg/kg daily of DHA plus 16 to 27 mg/kg daily of PPQ (EMOH, 2022) once daily for 3 days. However, if the patient experienced persistent vomiting, they were instead given artesunate intramuscularly or intravenously until the vomiting stopped. At that point, the patient was removed from the study and switched to oral therapy.

4.14. Enrollment and Follow-up

During enrolment, Health professionals thoroughly checked participants for signs and symptoms, including clinical malaria and other febrile illnesses. They also assessed for the presence of severe disease or any danger signs. If any of these conditions were found, the volunteers were treated according to the appropriate national guidelines, and if necessary, were referred to the next level of Healthcare. Pregnancy test ware conducted for volunteers of childbearing age (12-49 years), and those with a positive result were excluded. Hemoglobin levels were also measured using a spectrophotometer standard G6PD test™ analyzer device, and participants with levels below 7g/dl were excluded. If the participant met all the inclusion criteria, the health professionals completed the screening case reporting forms (CRFs). They also clearly explained the participant's appointment schedule and provided them with a visit card containing a personal identification number for future reference.

This study involved assessing enrolled individuals who had confirmed cases of *P. falciparum* malaria. The assessments included collecting a finger prick blood sample of approximately 500µl in EDTA coated microtainer tubes. A thin smear of the blood sample was used to verify the parasite species and count the number of parasites present.

A Dried Blood Spot (DBS) and whole blood were collected both on the day of enrollment and during follow-up days for parasite molecular studies. Follow-up clinical reassessments were conducted on specific days, including days 1, 2, 3, 7, 14, 21, and 28. Participants were advised to return to the health facility if they experienced chronic fatigue, abdominal pain, nausea and vomiting, or noticed changes in skin, eye, or urine color. Travel compensation was provided for all scheduled and unscheduled Follow-up day during the 28-day follow-up period.

In cases where participants missed a scheduled follow-up, the study team made efforts to reach them through home visits and phone communication. If participants missed their scheduled visit by one day early or one day late, they were still included in the analysis. However, if they were more than one day late in returning, they were considered withdrawn from the study and data was not collected.

4.15. Loss to Follow-up

All effort was made to schedule follow-up appointments for participants who did not return to the study, especially after they were administered the medication. Those patients who could not be located despite extensive efforts were categorized as lost to follow-up and subsequently censored or removed from the study. If an enrolled patient missed their follow-up visits and could not be found within the designated timeframe, this was categorized as a loss to follow-up, and they were not included in the treatment outcome assessment.

4.16. Patient Discontinuation or Withdrawal from the Study

Adhering to the protocols is crucial, and violations can occur either voluntarily or involuntarily. Voluntary violations occur when participants take antimalarial medications or related antibiotics. In contrast, involuntary violations often arise from circumstances beyond the participant's control, such as pregnancy, the onset of other illnesses, the identification of different Plasmodium species, or laboratory errors in parasitemia classification. Participants have the right to withdraw their consent at any time, and specific medical conditions like severe vomiting or failure to attend initial visit days may also result in withdrawal.

Serious adverse events can necessitate discontinuation of treatment, with the principal investigator tasked with assessing these events and ensuring they are properly documented. Participants who withdraw from the study will be monitored until they recover or until the study is completed;

however, they will be excluded from the analysis of treatment outcomes. All reasons for withdrawal will be meticulously documented to maintain accountability.

4.17. Assessment of Adverse Events

A serious adverse event refers to any incident that results in death, poses a life-threatening situation, necessitates hospitalization, causes lasting or notable disability, or requires specific medical or surgical action to avert serious consequences. Adverse events are defined as signs, symptoms, or unusual laboratory results that were absent at the time of enrollment but emerged during the follow-up day, or that were present at the time of D0 and worsened in severity during follow-up, even after the parasitemia had cleared. Clinical and physical examinations, along with questions on the standard list of adverse events linked to AL and malaria, were used to determine evaluations of adverse events. After the drug was administered, the caregiver was requested to report any strange occurrences (drug side effects, the child's tolerance to the treatment). At every stage, all clinical and demographic data were documented on a common record form.

4.18. Study Endpoints

The study endpoints were defined according to the revised WHO guidelines from 2009 and included: treatment failure (comprising early treatment failure, late clinical failure, and late parasitological failure), successful completion of follow-up without treatment failure (indicative of adequate clinical and parasitological responses), loss to follow-up, withdrawal from the study, and protocol violations.

4.19. Data Quality Assurance

Progress was monitored early in the study to assess the quality of screening, recruitment, randomization, and documentation practices. Systematic, independent audits were conducted during the course of the study to evaluate study conduct and compliance with the protocol, standard operating procedures (SOPs), good clinical practice, and the applicable regulatory requirements. Quality control checks were performed using positive and negative controls for Giemsa staining, pregnancy test, Microscopy, and PCR materials. We used the equipment that was up to date in their calibration.

The systems used to ensure the quality of every aspect of the study focused on subject protection and the reliability of study results.

4.20. Data Management

The principal investigator ensured that all data were accurately collected and entered into the case report form and that the study protocol was closely followed. Laboratory and clinical data were recorded daily on the case report form designed for the study. The data were entered into Research Electronic Data Capture (REDCap) in real time using tablets. Data obtained from the original documents had to match the original documents exactly, or any differences had to be justified. Any alterations or corrections made to a case report form were dated, and justified, and did not overwrite the initial information. The completeness of each case report form was checked. All data generated from the study were entered into the secure database of the knowledge management directorate of AHRI. Confidentiality of data was maintained; the paper forms were kept in a locked file cabinet at the knowledge management directorate of AHRI for five years after the completion of the study and the databases had standard password systems for all onsite and offsite backups. The principal investigators destroyed the CRFs after the end of the permitted storage time (5 years) at AHRI.

4.21. Data Analysis and Interpretation

Data were collected using standardized case report forms as recommended by the WHO. To minimize transcription errors, all questionnaires were entered twice into a designated database by two independent data clerks, utilizing REDCap. The data was then exported to Excel. Cleaned and validated data was analyzed using Stata 14 and R/RStudio version 4.4.2 with a significance level set at 5%. Descriptive statistics are presented using counts, percentages, means, medians, standard deviations, and ranges. The normality of the data was assessed with the Shapiro-Wilk test. Consequently, the non-parametric Kruskal-Wallis test was applied to determine group differences, and Dunn's test was utilized to identify variations in median groups. Efficacy data was assessed through two methods, namely, the Kaplan-Meier method and the per-protocol analysis method.

4.22. Dissemination of Results

The results of this study will be disseminated with relevant institutions, such as Addis Ababa University's College of Health Sciences, School of Medicine, Department of Microbiology Immunology and Parasitology, AHRI, and the study sites for future reference. Furthermore, a summary of the study will be submitted for publication in a peer-reviewed journal.

4.23. Ethical Consideration

An ethical clearance approval latter was obtained from the Addis Ababa University College of Health Science Department of Microbiology, Immunology, and Parasitology (Ref.No: DREC/001/2024) and Ministry of eduquetion (Ref. No. MoSHE 17/152/918/23) for the initiation of the study. No additional samples were collected from the participants specifically for this study. Written informed consent and assent were obtained from adult participants and the parents or guardians of children after explaining the study's purpose and objectives. Participants had the complete freedom to continue or withdraw from the study at any time. All data was secured using codes, with access restricted to the principal investigator.

Laboratory specimen labels and study reports excluded any mention of names. Identifiable information was securely stored in a locked metal cabinet, accessible exclusively by the study clinician or their appointed representative in their absence. All collected materials were assigned unique study identification numbers, ensuring no direct link to personal details.

5. RESULTS

5.1. Baseline Characteristics of the Study Participants

A total of 5, 006 participants experiencing fever were tested for malaria during the study period. Among these, 506 cases were positive for *Plasmodium* species (490 *P. falciparum*, 14 *P. vivax*, and 2 *P. falciparum* and *P. vivax* mixed infections). Of the malaria patients, 286 were males, while the rest were females. The study enrolled 91 patients who fulfilled the inclusion criteria and provided consent to participate. However, 16 participants were unable to complete it: 4 withdrew involuntarily due to persistent vomiting on day one, and 12 were lost to follow-up on days 7, 14 and 21 with the same participants (Figure 2). Among the 91 participants, 58 (63.7%) were male and the rest 33 (36.3%) were female. The median age of participants was 10 years and mean axillary body temperature at baseline was 38.3 °C (37-39.7). Before initiation of treatment, the median parasite count was 10372/µl (3624.5-24580) and the average hemoglobin level was 13.4 g/dl (Range: 7.4-18.4) as shown in table 1. In this study, 80.2% (73 participants) reported having their bed nets. Of those, 63% (46 participants) used them at night, whereas 35.6% (26 participants) did not use their bed nets.

Table 1: Baseline Characteristics of Study Population During the 28-Day Follow-Up Period of the Artemether-Lumefantrine Effectiveness Study in the Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

Population characteristics	**Age category**			
	Under 5(n=22)	5 to 15(n=44)	15(n=25)	Total
Median age(range)	4(3-4)	9.5(7-14)	25(19-36)	10(5-17)
Male (n, %)	16(72.7)	24(54.6)	18(72)	58(63.7)
Weight(Kg),Median(IQR)	13.5 (11-15)	24 (18.4-38.5)	52.1(11.9)	50(43-57)
Hemoglobin(g/dl),mean(SD)	11.9 (2)	13.4(2.4)	14.7(2)	13.4(2.2)
Mean Temp (ºC) (Range)	38.5(38.1-38.9)	38.3(38.1-38.4)	38.1(37.9-8.5)	38.3(0.7)
Median of microscopic Parasite density/ µl (IQR)	19660.3(5600-42260)	9838.5(4581-20591.5)	7226.5(2131.5-18120)	10372(3624.5-24580)
Median qPCR Parasite density/ µl (IQR)	31771.7(12882.71-155408.2)	15962.78(5175.24-30537.02)	2882.88(768.6-7502.28)	13391(2882.9-31304.7)

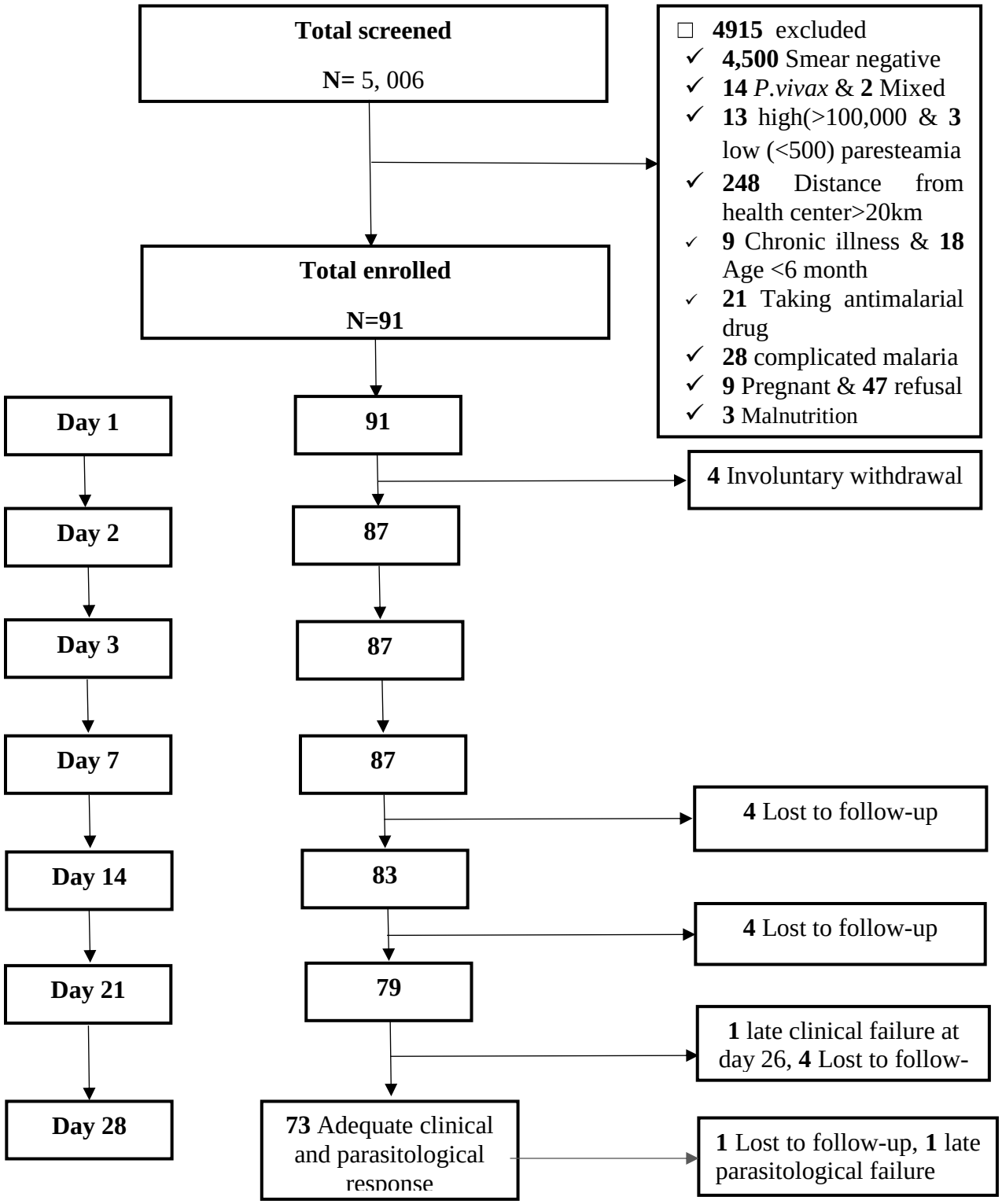

Figure 2: The flowchart outlining the enrollement and follow-up process of participants during the study period at Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

5.2. Treatment outcome

5.2.1. Cure rate of Artemether-Lumefantrine

Out of the 91 participants enrolled in the study, 73 successfully completed the follow-up period and demonstrated adequate clinical parasitological response (ACPR) by day 28. Within the 75 patients assessed for treatment outcomes, 2.7% (2 patients) were classified as treatment failures. This included one late clinical failure (LCF) recorded on day 26 and one late parasitological failure (LPF) on day 28. It was determined using PCR correction that there were no ETF, LCF and LPF (Annex 2). Based on the per-protocol analysis, the cure rate for AL among the study participants was 97.3% (95% CI: 89.7-99.4) without PCR correction, while it was 100% with PCR correction (Fig. 2 & Table 2.

Table 2:28-Day Cure Rate of Artemether-Lumefantrine in Treating Uncomplicated *P. falciparum* Malaria Cases at Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

Treatment outcome	**n(%)**	**95 % CI**
ETF	0(0.0)	-
LCF uncorrected	1(1.3)	0.034-7.2
LCF corrected	0 (0)	-
LPF uncorrected	1(1.3)	0.034-7.2
LPF corrected	0(0)	-
ACPR before PCR correction	73(97.3)	90-99.7
ACPR after PCR correction	73(100)	-
PP uncorrected cure rate	73/75 (97.3%)	89.7-99.4
PP corrected cure rate (95% CI)	73/73: (100%)	-
K-M uncorrected cure rate (95% CI)	97.4	93.8-100

K–M: Kaplan–Meier method, PP: per-protocol analysis

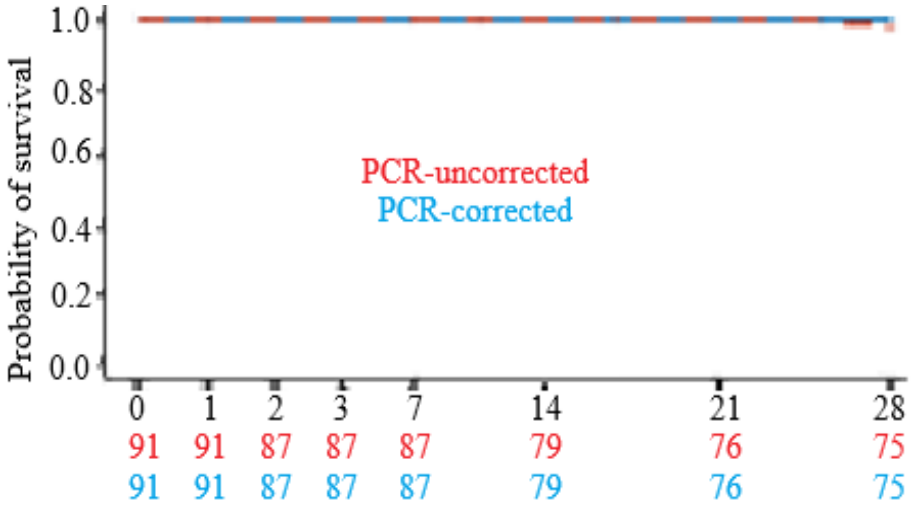

Figure 3: Kaplan–Meier survival curves, both PCR-uncorrected and PCR-corrected, assessing the outcomes of AL treatment for uncomplicated *P. falciparum* malaria in Asayita Primary Hospital, Northeast Ethiopia form December 2023 to March 2024

Table 3*:* Estimated amplicon size (bp) of the msp1 and msp2 alleles for participants with recurrent P. falciparum infection on day 0 and day of recurrence and primers and probes used in Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

		Sample Id			
		AA034-D0	AA034-D26	AA081-D0	AA081-D28
Msp1	Mad20_1	148.1	137.9	175.2	128.5
	Mad20_2	175.6	166.4	-	137.7
	K1_1	120.1	120.0	120.1	-
	K1_2	166.9	166.8	128.2	-
Msp2	3D7_1	228.7	264.6	249.8	-
	FC27_1	-	-	-	431.0
PFPK2	PfPk2_1	186.3	170.9	162	162
Result		**New infection**		**New infection**	

5.2.2. Clinical Outcome

On the recruitment day, all participants (100%, 91/91) were febrile, with axillary temperatures of at least 37.5 °C. This number dropped to 12.1% (11/91) by day 1, with no fever cases recorded beyond that point. As a result, the fever clearance rate was 87.9% (80/91) on day 1 and reached

100% (87/87) by day 2 (Table 4 and Fig. 4, A) and no febrile cases detected afterward except the one LCF case that was identified as reinfection after PCR genotyping results.

Hemoglobin levels were measured at intervals on days 0, 7, 14, 21, and 28, showing an upward trend in the average hemoglobin levels from the baseline to the end of the follow-up period. At baseline, 65.9% of study participants (60/91) were non-anemic, while 34.1% (31/91) were anemic. The mean hemoglobin levels among anemic study participants were 10.87 g/dl (range: 7.4-12.9 g/dl. A gradual increase in mean hemoglobin levels was observed throughout the study period. By day 7, the average hemoglobin concentration had increased to 10.9 g/dl, with a range of 8.2 to 16.3 g/dl. This improvement persisted, reaching 11.9 g/dl (range: 8.2–16.2 g/dl) by day 14. The positive trend continued, with the mean level rising to 12.4 g/dl (range: 8.4–17 g/dl) by day 21 and further increasing to 13.2 g/dl (range: 8.7–18 g/dl) by day 28. Indicating a statistically significant improvement in hemoglobin status and the findings highlight the dynamic nature of hemoglobin level.

Table 4: Fever and Parasite Clearance Rates in Study Population during Supervised Treatment and Follow-Up Period in Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

Variable	**Age group**	**Follow-up days**			
		D0(Baseline)	**D1(24hr)**	**D2(48hr)**	**D3(72hr)**
Parasitaemia detected, n (%)	(<5)	22(100)	20(90.9)	9(40.9)	3(13.6)
	(5-15)	44(100)	37(84.1)	10(24.4)	1(2.4)
	(>15)	25(100)	16(64)	0(0.0)	0(0.0)
	Total	91(100)	73(80.2)	19(21.8)	4(4.6)
Fever case (≥ 37.5, °C)	(<5)	22(100)	4(1 8.2)	(0.0)	0(0.0)
	(5-15)	44(100)	6(13.6)	0(0.0)	0(0.0)
	(>15)	25(100)	1(4)	0(0.0)	0(0.0)
	Total	91(100)	11(12.1)	0(0.0)	0(0.0)

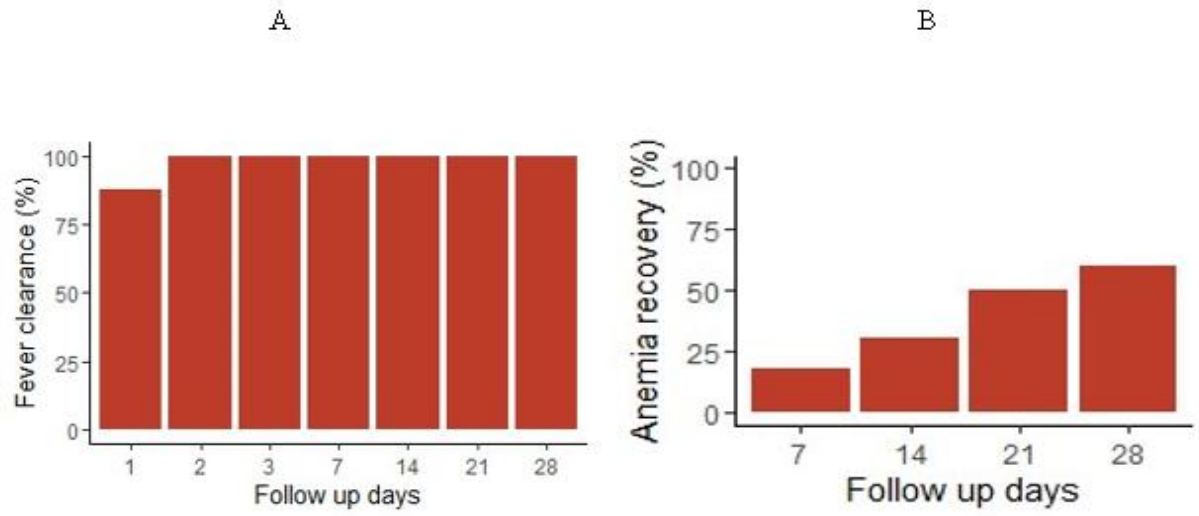

Figure 4: Fever clearance and anemia (hemoglobin) recovery over time in Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

5.2.3. Parasite Clearance

Based on microscopic examination, the parasitaemia detection rate was 80.2% (73/91) by day 1, and dropped to 21.8% (19/87) by day 2 and 4.6% (4/87) on day 3. Consequently, the rate of parasite clearance was significant, with 19.8% (18/91) of participants eliminating parasitemia by Day 1, 78.2% (68/87) by Day 2, and achieving a 95.4% clearance by Day 3. Four participants tested positive on day 3; however, the participants WHO tested positive on day 3 were followed to day 28 and had ACPR. Four school-aged children, three under-fives, and one seven-year-old showed microscopic positive by Day 3, with significant difference across the age groups. The median parasite density demonstrates significant variations when comparing day 0 with the follow-up days (p= 0.000). Moreover, a marked difference in the median parasite density is observed between day 1 and the subsequent follow-up days (p= 0.000). The qPCR analysis of the four study participants who tested positive by microscopy revealed that one participant was parasite-free by day 7, two participants were parasite-free by day 14, and all participants were parasite-free by day 21.

The average asexual parasite density differs significantly (p = 0.033) between the age groups of <5 and >15 years. This discrepancy suggests that, on average, parasitemia levels are higher in the <5-year age group. The higher average parasite density of *P. falciparum* malaria in under five

children, compared to those over fifteen, is largely due to the underdeveloped immune system in young children, which lacks the adaptive immunity found in older individuals. This makes young children more vulnerable to severe malaria infections and increased parasite loads. In contrast, older populations may develop immunological tolerance through repeated malaria exposures, resulting in lower parasite density.

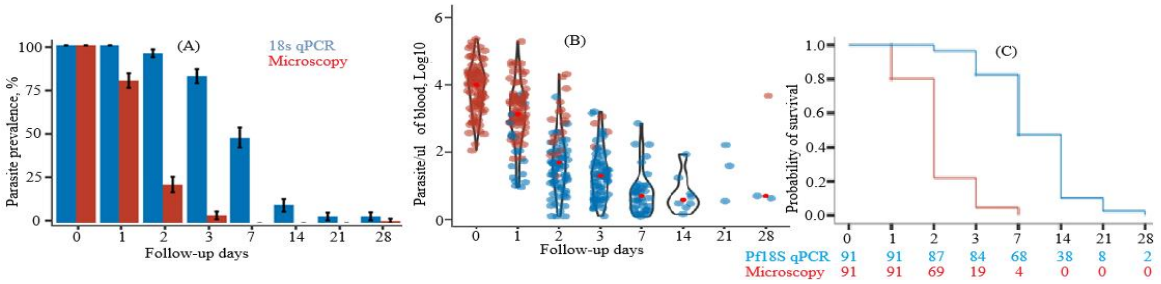

Figure 5: Parasite prevalence, dynamics of parasite density and Kaplan Meier survival estimation: A) Parasite prevalence during the follow-up period. B). the analysis of parasite density dynamics was performed using the Kruskal-Wallis and Dunn's tests to determine variations among median groups. C). Kaplan Meier survival estimation: Pattern of asexual parasite clearance in *P. falciparum* mono-infected patients treated with AL in Asayita Primary Hospital, Northeast Ethiopia. From December 2023 to March 2024

5.2.4. Gametocyte Clearance

The analysis on gametocyte clearance rates indicates significant progress in treatment effectiveness over a designated period. At the baseline observations showed a gametocyte prevalence of 7.69% on Day 0, which increased slightly to 8.79% on day 1, but then decreased to 6.90% by day 2. The count remained stable at 6 on Day 3, and notably, by Day 7, only 1.22% of cases displayed gametocytes, with no positive cases recorded after day 7.

5.2.5. Adverse Events Following AL Treatment

Data on adverse events (AE) associated with AL treatment was collected through self-reports/observations and documented in case reporting forms. Throughout the 28 day follow-up period, no serious AE were reported. Overall, there were 126 AE reported after the administration of the drug, with the most common being cough, headache, abdominal pain, diarrhea, weakness, fever, and dark urine. Most of the AE resolved with the elimination of asexual parasite, except for cough, headache, abdominal pain, and diarrhea as shown in Table 5.

Table 5: Clinical Signs and Symptoms Following Artemether-Lumefantrine Treatment in Asayita Primary Hospital, Northeast Ethiopia from December 2023 to March 2024

Follow-up day	Day-1 n(%)	Day-2 n(%)	Day-3 n(%)	Day-7 n(%)	Day-14 n(%)	Day-21 n(%)	Day-28 n(%)	Total
Fevers	2(2.2)	5(5.8)	2(2.3)	-	2(2.3)	-	2(2.3)	13
Chili	-	5(5.8)	2(2.3)	2(2.3)	-	-	-	9
Headache	1(1.1)	7(8.1)	7(8.1)	-	2(2.3)	2(2.3)	2(2.3)	21
Nausea	2(2.3)	2(2.3)	1(1.2)	-	-	-	-	5
Vomiting	2(2.2)	1(1.2)	1(1.2)	-	-	-	-	4
Abdominal pain	6(6.6)	5(5.8)	3(3.5)	3(3.5)	1(1.2)	-	-	18
Diarrhea	1(1.1)	1(1.2)	2(2.3)	3(3.5)	1(1.3)	-	-	8
Cough	2(2.2)	4(4.6)	3(3.5)	9(10.4)	4(4.6)	3(3.5)	1(1.2)	26
Weakness	4(4.4)	3(3.5)	2(2.3)	-	1(1.2)	-	-	10
Body ache	2(2.2)	-	-	-	-	-	-	2
Rash	-	1(1.2)	-	-	-	-	-	1
Mouth lesion	-	-	1(1.2)	-	-	-	-	1
Dark urine	6(6.6)	2(2.3)	-	-	-	-	-	8
Total	28	36	24	17	11	5	5	126

6. DISCUSSION

The World Health Organization emphasizes the importance of evaluating and monitoring first-line antimalarial drugs, such as AL treatment for malaria, every two years to ensure their continued effectiveness(WHO, 2009). Hence, this study aimed to assess the therapeutic effectiveness of the first-line antimalarial Artemether-lumefantrine in Aysaita Primary Hospital, Northeast Ethiopia. According to the per-protocol analysis, the cure rates of AL were found to be 97.3% without PCR correction and 100% with PCR correction. This finding is in line with the WHO-recommended cure rate cut-off value of 90% (WHO, 2009), suggesting the therapeutic effectiveness of AL in treating uncomplicated *P. falciparum* malaria in the study area. A similar high cure rate of AL was also reported in different transmission settings in Ethiopia; 98.6% in Arba Minch, South Ethiopia (Gubae *et al.*, 2023), 96% in Chewaka (Abamecha *et al.*, 2020), 98.3% in Northwest Ethiopia (Deressa *et al.*, 2017) and 97.3% Northwest Ethiopia (Woldesenbet *et al.*, 2024). The high PCR-corrected cure rate of AL is also comparable with findings from Niger (98.9%, 92.2% and 97.1% in Gaya, Tessaoua and Agadez, respectively) (Laminou *et al.*, 2024), and Northern Zambia (98.8%) (Ippolito *et al.*, 2020).

The PCR genotyping revealed that the two participants who tested positive on days 26 and 28 were reinfections. The recurrence of *P. falciparum* malaria may arise due to re-infection or recrudescence. The absence of treatment failure indicates that lumefantrine was an effective partner drug, supporting the use of AL as a first-line treatment in the study area (WHO, 2009). Treatment failures may occur due to age distribution, poor adherence, vomiting, drug resistance, substandard or suboptimal dosing, and inadequate drug absorption resulting in insufficient plasma concentrations (Baird, 2005, WHO, 2015). Artemether is rapidly absorbed and cleared from the body, with a half-life of about two hours, whereas lumefantrine shows inconsistent absorption and is eliminated at a slower rate (Baird, 2005, WHO, 2015). Lumefantrine, being a lipophilic substance, has inconsistent bioavailability unless taken with fatty foods. As a result, it is advised to take AL with a meal containing fats, like a small biscuit or milk (EMOH, 2022). This study did not determine adequate plasma concentrations of lumefantrine; however, all participants successfully completed the full treatment regimen, which was administered with a cream biscuit to enhance absorption.

The most common indication of malaria is fever or a recent history of fever, but this is not the peculiar presentation as there are many other febrile illnesses (Fitri *et al.*, 2022).. The use of paracetamol in febrile patients with uncomplicated malaria may contribute to rapid fever clearance by lowering the hypothalamic set-point temperature (Zhang *et al.*, 2021), which can complicate the interpretation of clinical efficacy outcomes. However, this rapid resolution of fever supports the effectiveness of AL in treating uncomplicated *P. falciparum* malaria within 48 hours. In this study, all participants were febrile at baseline. Of the participants who were febrile, 87.9% cleared fever on day 1, and all cleared fever by day 2. This outcome aligns with previous studies conducted in Ethiopia (Daka *et al.*, 2024, Tesfaye *et al.*, 2024, Woldesenbet *et al.*, 2024), indicative of continuing quick clinical and parasitological resolutions after AL treatment in the study area

Hemolytic anemia is a common complication of *P. falciparum* malaria, as the parasite infects RBCs at all stages, leading to their destruction and subsequent anemia(Bassey *et al.*, 2024). Effective treatment with AL clears the parasite, halting RBC destruction and allowing hematological recovery through resumed erythropoiesis. In this study, there was a slight increase in mean hemoglobin levels by day 7, 14, 21, followed by a significant rise by day 28. An individual patient data analysis from Sub-Saharan Africa also demonstrated the resolution of anemia and Hemoglobin levels after treatment (Zwang *et al.*, 2017). These findings are consistent with studies conducted in different regions in Ethiopia (Gubae *et al.*, 2023, Woldesenbet *et al.*, 2024).

The rapid and significant parasite clearance observed in this study is indicative of the potent antimalarial activity of AL. The parasite clearance rate on Day 3 was 95.4%, differing from the results of other studies conducted in Ethiopia (Daka *et al.*, 2024, Ebstie *et al.*, 2015, Gubae *et al.*, 2023) and Colombia (Olivera *et al.*, 2020) which documented full clearance by Day 3. However, it is consistent with the findings reported in other studies (Abamecha *et al.*, 2020, Woldesenbet *et al.*, 2024) and in other regions (Nhama *et al.*, 2021). In this study, all patients who tested positive on day 3 had high baseline parasite densities (60,563/µl, 63,160/µl, and 65,223/µl). However, one participant had a lower parasite density (30,124/µl,) than the other three participants. It has been noted that high initial parasitaemia or splenectomy, pharmacokinetics and pharmacodynamics, immunity, safety, and tolerability influence parasite clearance rates (Baird, 2005, WHO, 2020). Conversely, delayed parasite clearance, indicated by the day 3 positivity, may serve as a marker for artemisinin partial resistance (Mihreteab *et al.*, 2023). Studies conducted across various African

countries indicate the prevalence of artemisinin partial resistance on the continent (Uwimana *et al.*, 2021).

Molecular methods of parasite detection using DNA or RNA markers capable of detecting parasitemia at low level. These highly sensitive techniques show that persistent submicroscopic parasitemia may be common after treatment with AL(Homann *et al.*, 2017). After successful treatment of malaria, remnants of killed asexual parasites and either live or killed gametocytes can still release *Plasmodium* DNA, which may be detectable in patients who have been cured of the infection (Haanshuus and Mørch, 2020). In this study, PCR-determined submicroscopic *P. falciparum* positivity rates were 78.2% on day 3, 43.4% on day 7, 9.6% on day 14, 2.5 on day 21 following a full dose of AL, highlighting concerns about the persistence of submicroscopic infections and their potential role in ongoing transmission.

The finding of a significant difference in average asexual parasite density between the age groups of children under 5 and adults over 15 ($p = 0.033$), highlights the vulnerability of younger populations to parasitic infections. Studies have consistently shown that children tend to have higher rates of parasitemia due to factors such as underdeveloped immune systems. Furthermore, older individuals generally exhibit higher levels of acquired immunity, reducing their susceptibility to high-density infections (Baird, 2005, WHO, 2015).

Gametocytes were identified in some participants, likely as a result of the developmental properties of *P. falciparum* gametocytes. These gametocytes require 9 to 12 days to fully mature after the asexual parasites have formed, during which time they remain concealed in extravascular sites like the bone marrow and spleen until they reach maturity (Venugopal *et al.*, 2020). Artemisinin derivatives are known to have a gametocidal effect on the sexual stages of the parasite. In this research, gametocytes were cleared by day 7, consistent with findings from other studies conducted across different regions of Ethiopia (Gubae *et al.*, 2023, Woldesenbet *et al.*, 2024).

Artemether-lumefantrine was found to be the well-tolerated anti-malarial drug, demonstrating continued effectiveness and safety across all age groups of the participants. The majority of reported AE are mild to moderate intensity and are often associated with concomitant infections (Abamecha *et al.*, 2020, Gubae *et al.*, 2023, Teklemariam *et al.*, 2017, Woldesenbet *et al.*, 2024). The study did not report any serious AE and not realted AL. Most of the adverse events had

resolved promptly after parasite clearance. In this study, the most frequently reported adverse events after initiating the drug included cough, headache, abdominal pain, diarrhea, weakness, fever, and dark urine.

7. LIMITATIONS OF THE STUDY

The partial drug administration was the potential limitation in this study. However, the first dose was given in direct observation of a study nurse with instructions provided to participants or guardians. This study did not conduct plasma drug concentration analysis to verify drug absorption following the administration of AL. The use of non-stage-specific primers is another limitation of this study, as they detect all stages of the parasite, which can affect parasite clearance measurements and make it difficult to compare results with microscopy clearance. During the write-up of the proposal, we use the fourth edition of the national malaria guideline that indicates during the first trimester, AL is contraindicated due to this reason; pregnant women are excluded from the study, but the updated guideline indicates AL is administered at all trimesters. Another limitation of this study is the high number of participants lost to follow-up, primarily due to their nomadic lifestyle, which makes it challenging to track them. However, this loss is below the limits of the contingency plan.

8. CONCLUSION

Artemether-Lumefantrine has shown remarkable effectiveness in managing uncomplicated *P. falciparum* malaria within the study area. It demonstrates rapid fever resolution, asexual parasite and gametocyte clearance, along with a significant improvement in mean hemoglobin levels post-treatment. Moreover, AL's excellent safety and tolerability are evident, with no serious adverse events reported. These findings strongly support the continued use of AL as the primary treatment for uncomplicated *P. falciparum* malaria.

RECOMMENDATIONS

The effectiveness of AL requires thorough monitored to detect potential treatment failures, which may result from drug resistance or inadequate adherence. To ensure its effectiveness, it is important to conduct ongoing therapeutic studies at sentinel sites throughout both the region and the country. Ongoing healthcare education for patients on the importance of completing the full dose of AL treatment is essential. This strategy supports the prevention of drug resistance while enhancing the success of treatment outcomes.

REFERENCES

Abamecha, A, Yilma, D, Addisu, W, El-Abid, H, Ibenthal, A, Noedl, H, *et al.* 2020. Therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Chewaka District, Ethiopia. *Malaria Journal,* 19, https://doi.org/10.1186/s12936-020-03307-4.

Alemayehu, AA, Castaneda-Mogollon, D, Tesfa, H, Getie, S, Mohon, AN, Balasingam, N, *et al.* 2021. Expansion of the Plasmodium falciparum Kelch 13 R622I mutation in Northwest Ethiopia. *Research square,* 1, https://doi.org/10.21203/rs.3.rs-171038/v1.

Assefa, A, Kassa, M, Tadese, G, Mohamed, H, Animut, A & Mengesha, T 2010. Therapeutic efficacy of Artemether/Lumefantrine (Coartem(R)) against Plasmodium falciparum in Kersa, South West Ethiopia. *Parasit Vectors,* 3**,** 1.

Ayalew, MB 2017. Therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Ethiopia: a systematic review and meta-analysis. *Infect Dis Poverty,* 6**,** 157.

Baird, JK 2005. Effectiveness of antimalarial drugs. *New England Journal of Medicine,* 352**,** 1565-1577.

Bartoloni, A & Zammarchi, L 2012. Clinical aspects of uncomplicated and severe malaria. *Mediterr J Hematol Infect Dis,* 4**,** e2012026.

Bassey, BE, Etefia, EV, Adams, EG & Akpan, ME 2024. Prevalence of malaria, the effect of parasitaemia on blood parameters and iron level of infected children under 5 years of age in Itu, South-South, Nigeria. *FUDMA JOURNAL OF SCIENCES,* 8**,** 119-129.

Bayih, AG, Getnet, G, Alemu, A, Getie, S, Mohon, AN & Pillai, DR 2016. A Unique Plasmodium falciparum K13 Gene Mutation in Northwest Ethiopia. *Am J Trop Med Hyg,* 94**,** 132-5.

Belay, B 2018. Four year trend analysis of Malaria prevalence in Aysaita primary Hospital, Aysaita woreda, Afar regional state, Northeast Ethiopia https://etd.aau.edu.et/items/663f1f98-81e8-471f-8dc6-e5315ea080c2

Bharti, PK, Shukla, MM, Ringwald, P, Krishna, S, Singh, PP, Yadav, A, *et al.* 2016. Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria from three highly malarious states in India. *Malar J,* 15**,** 498.

Conrad, MD, Asua, V, Garg, S, Giesbrecht, D, Niaré, K, Smith, S, *et al.* 2023. Evolution of partial resistance to artemisinins in malaria parasites in Uganda. *New England Journal of Medicine,* 389**,** 722-732.

Daka, D, Woldeyes, D, Golassa, L, Alemayehu, GS, Zewde, Z, Tamiru, G, *et al.* 2024. Therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Arba Minch Zuria District, Gamo Zone, Southwest Ethiopia. *Malaria Journal* 23**,** 282.

Derbie, A, Mekonnen, D, Adugna, M, Yeshitela, B, Woldeamanuel, Y, Abebe, T, *et al.* 2020. Therapeutic Efficacy of Artemether-Lumefantrine (Coartem®) for the Treatment of Uncomplicated Falciparum Malaria in Africa: A Systematic Review. *Journal of Parasitology Research,* 2020**,** 1-14.

Deressa, T, Seid, ME, Birhan, W, Aleka, Y & Tebeje, BM 2017. In vivo efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria in Dembia District, northwest Ethiopia. *Ther Clin Risk Manag,* 13**,** 201-206.

Dimbu, PR, Horth, R, Candido, ALM, Ferreira, CM, Caquece, F, Garcia, LEA, *et al.* 2021. Continued Low Efficacy of Artemether-Lumefantrine in Angola in 2019. *Antimicrob Agents Chemother,* 65.

Ebstie, YA, Zeynudin, A, Belachew, T, Desalegn, Z & Suleman, S 2015. Assessment of therapeutic efficacy and safety of artemether-lumefantrine (Coartem(R)) in the treatment of uncomplicated Plasmodium falciparum malaria patients in Bahir Dar district, Northwest Ethiopia: an observational cohort study. *Malar J,* 14**,** 236.

EMOH 2022. Malaria Program Guideline, Fifth Edition. Addis Ababa, Ethiopia, August 2022.

Fitri, LE, Widaningrum, T, Endharti, AT, Prabowo, MH, Winaris, N & Nugraha, RYB 2022. Malaria diagnostic update: From conventional to advanced method. *Journal of Clinical Laboratory Analysis,* 36**,** e24314.

FMOH 2018. National malaria guidline-2018.Addis Ababa March 2018 fourth edition.

FMOH. 2021. *Manual for the laboratory diagnosis of malaria* https://www.researchgate.net/publication/356064987_MANUAL_FOR_THE_LABORATORY_DIAGNOSIS_OF_MALARIA

Fola, AA, Feleke, SM, Mohammed, H, Brhane, BG, Hennelly, CM, Assefa, A, *et al.* 2023. Plasmodium falciparum resistant to artemisinin and diagnostics have emerged in Ethiopia. *Nature Microbiology,* 8**,** 1911-1919.

Getnet, G, Fola, AA, Alemu, A, Getie, S, Fuehrer, HP & Noedl, H 2015. Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in Enfranze, north-west Ethiopia. *Malar J,* 14**,** 258.

Ghimire, P, Rijal, KR, Kafle, C, Karki, BS, Singh, N, Ortega, L, *et al.* 2018. Efficacy of artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in Nepal. *Trop Dis Travel Med Vaccines,* 4**,** 9.

Gubae, K, Mohammed, H, Sime, H, Hailgiorgis, H, Mare, AK, Gidey, B, *et al.* 2023. Safety and therapeutic efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria at Shecha health centre, Arba Minch, Ethiopia. *Malaria Journal,* 22**,** 9.

Haanshuus, CG & Mørch, K 2020. Detection of remaining Plasmodium DNA and gametocytes during follow up after curative malaria treatment among returned travellers in Norway. *Malaria Journal,* 19**,** 1-6.

Haendiges, J, Timme, R, Kastanis, G & Balkey, M 2020. Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit.

Hermsen CC, Telgt DS, Linders EH, van de Locht LA, Eling WM, Mensink EJ, Sauerwein RW. Detection of Plasmodium falciparum malaria parasites in vivo by real-time quantitative PCR. Mol Biochem Parasitol. 2001 Dec;118(2):247-51. doi: 10.1016/s0166-6851(01)00379-6. PMID: 11738714.

Homann, MV, Emami, SN, Yman, V, Stenström, C, Sondén, K, Ramström, H, *et al.* 2017. Detection of malaria parasites after treatment in travelers: a 12-months longitudinal study and statistical modelling analysis. *EBioMedicine,* 25**,** 66-72.

Ippolito, MM, Pringle, JC, Siame, M, Katowa, B, Aydemir, O, Oluoch, PO, *et al.* 2020. Therapeutic Efficacy of Artemether-Lumefantrine for Uncomplicated Falciparum Malaria in Northern Zambia. *Am J Trop Med Hyg,* 103**,** 2224-2232.

Karunajeewa, HA & Mueller, I 2016. How important is gametocyte clearance after malaria therapy? *BMC Med,* 14**,** 93.

Koko, VS, Warsame, M, Vonhm, B, Jeuronlon, MK, Menard, D, Ma, L, *et al.* 2022. Artesunate-amodiaquine and artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in Liberia: in vivo efficacy and frequency of molecular markers. *Malar J,* 21**,** 134.

Krishna, S, Mishra, S, Tiwari, P, Vishwakarma, AK, Khandai, S, Shrivastava, S, *et al.* 2021. Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in four malaria endemic states of India. *Malar J,* 20**,** 229.

Laminou, IM, Issa, I, Adehossi, E, Maman, K, Jackou, H, Coulibaly, E, *et al.* 2024. Therapeutic efficacy and tolerability of artemether-lumefantrine for uncomplicated Plasmodium falciparum malaria in Niger, 2020. *Malar J,* 23**,** 144.

Li, G, Yuan, Y, Zheng, S, Lu, C, Li, M, Tan, R, *et al.* 2022. Artemisinin-piperaquine versus artemether-lumefantrine for treatment of uncomplicated Plasmodium falciparum malaria in Grande Comore island: an open-label, non-randomised controlled trial. *Int J Antimicrob Agents,* 60**,** 106658.

Mihreteab, S, Platon, L, Berhane, A, Stokes, BH, Warsame, M, Campagne, P, *et al.* 2023. Increasing prevalence of artemisinin-resistant HRP2-negative malaria in Eritrea. *New England Journal of Medicine,* 389**,** 1191-1202.

Moriarty, LF, Nkoli, PM, Likwela, JL, Mulopo, PM, Sompwe, EM, Rika, JM, *et al.* 2021. Therapeutic Efficacy of Artemisinin-Based Combination Therapies in Democratic Republic of the Congo and Investigation of Molecular Markers of Antimalarial Resistance. *Am J Trop Med Hyg,* 105**,** 1067-1075.

Nhama, A, Nhamussua, L, Macete, E, Bassat, Q, Salvador, C, Enosse, S, *et al.* 2021. In vivo efficacy and safety of artemether-lumefantrine and amodiaquine-artesunate for uncomplicated Plasmodium falciparum malaria in Mozambique, 2018. *Malar J,* 20**,** 390.

Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg. 2007 Dec;77(6 Suppl):181-92. PMID: 18165491.

Ogutu, B 2013. Artemether and lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in sub-Saharan Africa. *Expert opinion on pharmacotherapy,* 14**,** 643-654.

Olivera, MJ, Guerra, AP, Cortes, LJ, Horth, RZ, Padilla, J, Novoa, J, *et al.* 2020. Artemether-Lumefantrine Efficacy for the Treatment of Uncomplicated Plasmodium falciparum Malaria in Choco, Colombia after 8 Years as First-Line Treatment. *Am J Trop Med Hyg,* 102**,** 1056-1063.

Owoloye, A, Olufemi, M, Idowu, ET & Oyebola, KM 2021. Prevalence of potential mediators of artemisinin resistance in African isolates of Plasmodium falciparum. *Malaria journal,* 20**,** 1-12.

Plewes, K, Turner, GDH & Dondorp, AM 2018. Pathophysiology, clinical presentation, and treatment of coma and acute kidney injury complicating falciparum malaria. *Curr Opin Infect Dis,* 31**,** 69-77.

Plucinski, M.M., Dimbu, P.R., Macaia, A.P., Ferreira, C.M., Samutondo, C., Quivinja, J.,*et al.* 2017. Efficacy of artemether–lumefantrine, artesunate–amodiaquine, and dihydroartemisinin–piperaquine for treatment of uncomplicated Plasmodium falciparum malaria in Angola, 2015. *Malaria journal*, *16*, pp.1-10.

Rivas-Morello, B, Horemans, D, Viswanathan, K, Taylor, C, Blanchard, A, Karamagi, H, *et al.* 2023. Assessing capacities and resilience of health services during the COVID-19 pandemic: lessons learned from use of rapid key informant surveys. *Frontiers in Public Health,* 11**,** 1102507.

Roux, AT, Maharaj, L, Oyegoke, O, Akoniyon, OP, Adeleke, MA, Maharaj, R, *et al.* 2021. Chloroquine and Sulfadoxine-Pyrimethamine Resistance in Sub-Saharan Africa-A Review. *Frontiers in Genetics* 12**,** 668574.

Sa, JM, Kaslow, SR, Krause, MA, Melendez-Muniz, VA, Salzman, RE, Kite, WA, *et al.* 2018. Artemisinin resistance phenotypes and K13 inheritance in a Plasmodium falciparum cross and Aotus model. *Proc Natl Acad Sci U S A,* 115**,** 12513-12518.

Sato, S 2021. Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology. *J Physiol Anthropol,* 40**,** 1.

St. Laurent, B, Miller, B, Burton, TA, Amaratunga, C, Men, S, Sovannaroth, S, *et al.* 2015. Artemisinin-resistant Plasmodium falciparum clinical isolates can infect diverse mosquito vectors of Southeast Asia and Africa. *Nature communications,* 6, https://doi.org/10.1038/ncomms9614**,** 8614.

Tedla, M 2019. A focus on improving molecular diagnostic approaches to malaria control and elimination in low transmission settings: Review. *Parasite Epidemiol Control,* 6**,** e00107.

Teklemariam, M, Assefa, A, Kassa, M, Mohammed, H & Mamo, H 2017. Therapeutic efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria in a high-transmission area in northwest Ethiopia. *PLoS One,* 12**,** e0176004.

Tesfaye, M., Assefa, A., Hailgiorgis, H. *et al.* Therapeutic efficacy and safety of artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria treatment in Metehara, Central-east Ethiopia. *Malar J* **23**, 184 (2024). https://doi.org/10.1186/s12936-024-04991-2

Uwimana, A, Legrand, E, Stokes, BH, Ndikumana, J-LM, Warsame, M, Umulisa, N, *et al.* 2020. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. *Nature medicine,* 26**,** 1602-1608.

Uwimana, A, Umulisa, N, Venkatesan, M, Svigel, SS, Zhou, Z, Munyaneza, T, *et al.* 2021. Association of Plasmodium falciparum kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *The Lancet Infectious Diseases,* 21**,** 1120-1128.

Venugopal, K, Hentzschel, F, Valkiunas, G & Marti, M 2020. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nat Rev Microbiol,* 18**,** 177-189.

Wampfler, R, Mwingira, F., Javati, S., Robinson, L., Betuela, I., Siba, P., Beck, H.P., Mueller, I., and Felger, I. 2013. Wampfler, R., Mwingira, F., JavaStrategies for detection of Plasmodium species gametocytes. PLoS One 8, e76316. 8.

White, NJ, Van Vugt, M & Ezzet, FD 1999. Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. *Clinical pharmacokinetics,* 37**,** 105-125.

WHO 2015. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

WHO 2020. Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010– 2019). Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

WHO 2022. World malaria report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.

WHO 2023a. WHO Guidelines for malaria, 14 March 2023. Geneva: World Health Organization; 2023 (WHO/UCN/GMP/ 2023.01). License: CC BY-NC-SA 3.0 IGO. .

WHO 2023b. World malaria report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

WHO 2024a. World Health Organization (31 October 2024). Disease Outbreak News; Malaria in Ethiopia.

WHO 2024b. World malaria report 2024: addressing inequity in the global malaria response. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.

WHO 2009. Methods for surveillance of antimalarial drug efficacy, ISBN 978 92 4 159753 1.

Wogu, MN & Nduka, FO 2018. Evaluating Malaria Prevalence Using Clinical Diagnosis Compared with Microscopy and Rapid Diagnostic Tests in a Tertiary Healthcare Facility in Rivers State, Nigeria. *J Trop Med*, 2018**,** 3954717.

Woldesenbet, D, Birhanie, M, Abere, A, Zeleke, AJ, Bezabih, MK, Semaw, M, *et al.* 2024. Therapeutic efficacy and safety of artemether-lumefantrine combination therapy for the treatment of uncomplicated Plasmodium falciparum malaria at Teda Health Centre, Northwest Ethiopia, 2022/23. *Malaria Journal,* 23**,** 266.

Zhang, M, Wang, C, Oberstaller, J, Thomas, P, Otto, TD, Casandra, D, *et al.* 2021. The apicoplast link to fever-survival and artemisinin-resistance in the malaria parasite. *Nature communications,* 12**,** 4563.

Zhou, G, Hemming-Schroeder, E, Gesuge, M, Afrane, YA, Lee, MC, Atieli, HE, *et al.* 2020. Gaps between Knowledge and Malaria Treatment Practices after Intensive Anti-Malaria Campaigns in Western Kenya: 2004-2016. *Am J Trop Med Hyg,* 102**,** 1358-1365.

Zwang, J, D'Alessandro, U, Ndiaye, J-L, Djimdé, AA, Dorsey, G, Mårtensson, AA, *et al.* 2017. Haemoglobin changes and risk of anaemia following treatment for uncomplicated falciparum malaria in sub-Saharan Africa. *BMC infectious diseases,* 17**,** 1-10.

ANNEXS

Annex 1: Significance of Parasite Clearance between Follow-up days

Day of follow-up		P-value	Day of follow-up		P-value
Days			Days		
Day 0	Day 1	0.000	Day 2	Day 7	0.218
	Day 2	0.000		Day 14	0.218
	Day 3	0.000		Day 21	0.218
	Day 7	0.000		Day 28	0.324
	Day 14	0.000	Day 3	Day 7	1.000
	Day 21	0.000		Day 14	1.000
	Day 28	0.000		Day 21	1.000
Day 1	Day 2	0.000		Day28	1.000
	Day 3	0.000	Day 7	Day 14	1.000
	Day 7	0.000		Day 21	1.000
	Day 14	0.000		Day 28	1.000
	Day 21	0.000	Day 14	Day 21	1.000
	Day 28	0.000		Day 28	1.000
Day 2	Day 3	0.532	Day 21	Day 28	1.000

Annex 2: The primers and probes used were specific for the 18S rRNA gene of both *P. falciparum* and *P. vivax*

Primers and probes sequences	
Pf18S FW	GTAATTGGAATGATAGGAATTTACAAGGT
Pf18S RV	TCAACTACGAACGTTTTAACTGCAAC
Pf18S probe	FAM-AACAATTGGAGGGCAAG-MGBNFQ
Pv18S FW	AGCAGCCGCGGTAATTCCA
Pv18S RV	ATGCGCACAAAGTCGATACGAAG
Pv18S probe	TEXASRED-AGCAACGCTTCTAGCTTAATCCACAT-BHQ2

The amplification was carried out under standard thermal cycling conditions, and the data were analyzed by comparing the cycle threshold (Ct) values of the samples to the standard curves.

Annex 3: **Symptoms of severe malaria**

Severe manifestation of *P. falciparum* malaria in adults and children (WHO, 2009).

Clinical manifestations

- Prostration
- Impaired consciousness,
- Respiratory distress (metabolic acidosis),
- Multiple convulsions,
- Circulatory collapse,
- Pulmonary oedema (radiological),
- Abnormal bleeding,
- Jaundice,
- Haemoglobinurea.

Laboratory findings

- Severe anaemia (haemoglobin<5g/dl, haematocrit<15%),
- Hypoglycaemia (blood glucose<2.2mmol/lor40mg/dl),
- Acidosis (plasma bicarbonate<15mmol/l),
- Hyperlactataemia (venous lactic acid > 5 mmol/l),
- Hyperparasitaemia (> 5% infected erythrocytes in non-immune patients),
- Renal impairment (serum creatinine above normal range for age)

Classification of severe malaria in children

Group 1: children at increased risk for death

- Prostration,
- Respiratory distress.

Group 2: children at risk for clinical deterioration

- Haemoglobin< 5 g/dl, haematocrit< 15%,
- Two or more convulsions within 24h.

Group3: Children with persistent vomiting

Annex 4: Weight-based administration of AL and assessment of drug administration at home

Weight (Age)	20mg of Artemether and 120mg of Lumefantrine (Coartem □)					
	0 hours	8 hours	24 hours	36 hours	48 hours	60 hours
5-14 kg (<3yr)	1 (tablet)	1	1	1	1	1
15-24 kg (3-9yr)	2	2	2	2	2	2
25-34 kg (9-14yr)	3	3	3	3	3	3
>34kg (>14yr)	4	4	4	4	4	4

1= successfully took medication

2= Vomited consquitive two doses

3= did not take the medication

Medications (with antimalarial activity) that should not be used during the study period

Annex 5: Medications that should not be used during the study period

- ✓ Antibiotics: tetracycline*, doxycycline, erythromycin, azithromycin, clindamycin, rifampicin, trimethoprim;
- ✓ Amodiaquine
- ✓ Artemisinin and its derivatives (artemether, artesunate, dihydroartemisinin)
- ✓ Atovaquone
- ✓ Chloroquine
- ✓ Chlorproguanil
- ✓ Dapsone
- ✓ Halofantrine
- ✓ Lumefantrine
- ✓ Mefloquine
- ✓ Naphthoquinone
- ✓ Pentamidine
- ✓ Piperaquine
- ✓ Primaquine
- ✓ Proguanil
- ✓ Pyrimethamine
- ✓ Pyronaridine
- ✓ Quinidine
- ✓ Quinine , Sulfamethoxazole
- ✓ Sulfadoxine, Sulfalene
- ✓ *Tetracycline eye ointments can be used.

Annex 6: Case screening form

Case screening form(Form 1) Study nurse

Study code_____ Date of visit:_____/_____/_____GC (day / month / year)Card No: _____

Health center/hospital: _____

Demographic data/past medical history

Participant name: _____

Sex: ☐Male ☐Female Date of birth____/____/_____ Age: ☐Above 6 months but less than 1 year
☐ 1 year and aboveAge:___in year

Will you live in this area for at least 4 weeks? ☐Yes ☐No (**If no, subject is not eligible.)**

Are you living within 20 km distance from this health centre/hospital? ☐Yes ☐No**(If home is more than 20 km from health center/hospital, subject is not eligible.)** Mobile phone #:_____☐N/A

Thick and thin blood smears for parasite confirmation (from finger stick #1)

Species by microscopy (only on day 0): ☐*P. falciparum* ☐*P.vivax* ☐Mixed (Pf + Pv) ☐Negative
Parasite density: _____

Lab diagnosis of *P. falciparum* monoinfection? ☐Yes ☐No (**If mixed or other infection, subject is not eligible.**)

Weight, Height and Temperature

Weight: ☐☐☐.☐kg Height: ☐☐☐cm Temperature (axillary):_____/_____/._____˚C

Past Medical History

History of fever in previous 24 h? ☐Yes ☐ No

If female 12-49 years: Are you pregnant? ☐Yes ☐No ☐Not sure **(If yes, subject is not eligible.)**

Are you breastfeeding? ☐Yes ☐No **(If yes, subject is not eligible.)**

ɐ you ever had a serious or chronic medical illness? ☐Yes ☐No **(If yes, subject is not eligible.)If yes,** what illnesses? ☐heart disease ☐kidney disease ☐liver disease
HIV/AIDS ☐other _____

Have you ever had an allergy or sensitivity to artemether-lumefantrime? ☐Yes ☐No **(If yes, subject is not eligible.)**

Have you ever had hemolysis or severe anemia? ☐Yes ☐No **(If yes, subject is not eligible.)**

Symptom assessment

Seizures?	☐Yes	☐No	Inability to sit/stand?	☐Yes	☐ No
Vomiting everything?	☐Yes	☐No	Not eating anything	☐Yes	☐No
Lethargy?	☐Yes	☐No	Discolored urine?	☐Yes	☐No

Inclusion criteria

Slide-confirmed mono-infection with *P. falciparum*	☐Yes	☐No
Age ≥ 6 months	☐Yes	☐No
Weight ≥ 5 kg	☐Yes	☐No
Permanent resident within 20 km of the enrolling health facility	☐Yes	☐No
Axillary temperature ≥ 37.5˚C	☐Yes	☐No
history of fever during the previous 24 hours	☐Yes	☐No
Can swallow medication	☐Yes	☐No
Able and willing to comply with study protocol for study duration	☐Yes	☐No

(If subject answers NO to any of the above questions, subject is not eligible.)		
Exclusion criteria		
Pregnant or breastfeeding	☐ Yes	☐ No
Serious or chronic medical illnesses	☐ Yes	☐ No
Current febrile illnesses not due to malaria	☐ Yes	☐ No
History of allergy/sensitivity to study drug	☐ Yes	☐ No
Danger signs or symptoms of severe malaria such as prostration, rapid shallow breathing, multipleseizures, shock, jaundice, hyperpyrexia	☐ Yes	☐ No
Refusal to take pregnancy test for woman (12-49 years)	☐ Yes ☐ No Taking regular	
medication which may interfere with antimalarial pharmacokinetics or efficacy	☐ Yes ☐ No Children	
weighing less than 5 kilogram	☐ Yes	☐ No
Severe malnutrition in child aged between 6-59 months as defined by presence of symmetrical edema involving at least the feet or a mid-upper arm circumference < 115 mm	☐ Yes	☐ No
(If subject answers YES to any of the above questions, subject is not eligible.)		
Patient informed consent and assent		
If subject is still eligible, perform informed consent (for all) Consent form signed/in chart: ☐ Yes ☐ No Consent for storage of blood sample: ☐ Yes ☐ No		
If consent or assent refused, why? (If refused, subject is not eligible.)		
Laboratory evaluation (copy from lab request form)		
Hemocue results		
Hemoglobin: ☐☐.☐g/dl (If Hb < 7 g/dL, subject is not eligible.)		
Pregnancy test		
Result of urine pregnancy test (females 12-49 year only): ☐ Positive ☐ Negative	☐N/A	☐ Refused
(If positive or refused, subject is not eligible.)		
REPEAT thick and thin parasite smear		
Repeat slide obtained? ☐ Yes	☐ No If not, why not?	
P. falciparum infection confirmed	☐Yes ☐ No (If NO,	
subject is not eligible. Refer for further care.)		
RDT result		
Please re-check species by rapid test (only on day 0).	☐ *P. falciparum* ☐P.vivax	
☐Mixed (Pf + Pv)	☐Negative	
Laboratory exclusion criteria (for review by enroller)		
Severe anemia, defined as Hg < 7 g/dl	☐ Yes	☐ No
Positive pregnancy test	☐ Yes	☐ No
Any Plasmodium other than *P. falciparum* monoinfection	☐ Yes	☐ No
Does the subject meet any above laboratory exclusion criteria? ☐ Yes ☐ No (If yes, subject is not eligible.) If subject is still eligible after lab evaluation, proceed to enrolment form.		
Subject identity number assignment		
After consenting, place Day 0 label here. Tear off all corresponding subject ID labels and affix to this form. Attach a "Visit 0" label to the lab request form and send the subject to the lab for further evaluation.	Subject ID number label	

Study staff name:_____Signature: _____

Annex 7: Laboratory request form

Laboratory request form (Form 3) Study Laboratory		
Study ID: _____ Card No_____ Date of visit: __ __ / __ __ / __ __ __ __EC (day / month / year)	Subject ID number label	Study arm: AL
Laboratory technologist		

Place label in lab log book. Write all results in spaces below AND in laboratory log book.

1. Hemoglobin: ☐☐.☐ g/dL

2. Parasitemia:

Are *P. falciparum* asexual parasites present? ☐Yes ☐ No

If yes, parasite density (parasites/µL): ☐☐☐☐

Are *P. falciparum* gametocytes present? ☐ yes ☐ No

Are species other than *falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ P. vivax ☐ P. Ovale ☐ P. malariae

3. Urine pregnancy test (women aged 12-49 years): ☐ Positive ☐ Negative ☐ N/A

4. Filter paper for parasite molecular test (Whatmann 3 MM) ☐ Yes ☐ N

5. Cell pellet in RNA protect ☐ Yes ☐No

6. Please re-check species by rapid test (only on day 0). ☐ *P. falciparum* ☐ *P.vivax* ☐ Negative

NB: Hb measurement on days 0, 7, 14, 21, 28 and any other day.

Urine test and RDT on day 0 only.

Slides, DBS, and cell pellet to be collected throughout the follow-up schedule and unscheduled days.

Lab personnel signature: _____ Date: __ __ / __ __ / __ __GC

Annex 8: Case Report Form

<table>
<tr><td colspan="6">Case report form: day 0/enrolment (Form 4) Study HO/nurse</td></tr>
<tr><td colspan="2">Study ID_____
Date of visit: __ __ / __ __ / __ __ __ __GC(day / month / year)</td><td colspan="3">Subject ID
Place an additional subject identity number label in the lab log book.</td><td>Study arm: AL</td></tr>
<tr><td colspan="6">Laboratory summary (HO)</td></tr>
<tr><td colspan="6">Copy lab results from laboratory request form:
Hemoglobin:☐☐.☐ g/dl
Result of urine pregnancy test (females 12-49 year only) : ☐ Positive ☐ Negative ☐N/A</td></tr>
<tr><td colspan="6">Malaria prevention data (HO)</td></tr>
<tr><td colspan="6">Does the subject's household own a bednet? ☐Yes ☐ No
Did the patient sleep under a bednet last night? ☐ Yes ☐ No
Was the subject's home sprayed with insecticide in the past 12 months? ☐Yes ☐No ☐Not sure</td></tr>
<tr><td colspan="6">Baseline symptom data (HO)</td></tr>
<tr><td colspan="6">Presence of danger signs or signs of sever or complicated malaria? ☐Yes ☐No</td></tr>
<tr><td colspan="6">Recent medication (HO)</td></tr>
<tr><td colspan="6">Report all meds, including natural remedies and homeopathic medicines, taken within the previous 14 days.</td></tr>
<tr><td colspan="6">Have you taken any antimalarial medication in the past 14 days? ☐ Yes ☐No
Have you taken any other medication in the past 14 days? ☐ Yes ☐ No
If yes, give the names and dates of taking the medication.
Complete either the stop date or the 'ongoing' box.</td></tr>
<tr><td>Generic medicine name</td><td>Dates</td><td>Ongoing (Yes =☐</td><td>Total daily dose and unit</td><td>Route of administration</td><td>Indication for use</td></tr>
<tr><td></td><td>Start:
Stop:</td><td>☐</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">Physical exam (HO)</td></tr>
<tr><td colspan="6">Axillary Temperature (ºC): ☐☐.☐ Pulse (heartbeats/min): ☐☐☐</td></tr>
</table>

Respiratory rate (breaths/min): ☐☐☐				
Organ system			Normal?	If abnormal, describe finding
HEENT (MMM, evidence of OM)			☐ Yes ☐ No	
Cardiorespiratory			☐ Yes ☐No	
Signs of severe malnutrition (wasting, pedal edema)			☐ Yes ☐ No	
Petechial rash or jaundice			☐Yes ☐ No	
Mental status			☐ Yes ☐ No	
Other			☐ Yes ☐ No	
Did the exam reveal any febrile conditions caused by other diseases OR evidence of severe malnutrition? ☐ Yes ☐No (If yes, the subject is not eligible.)				
Medication administration (nurse)				
Send subject to nurse for medication administration. Subject weight:☐☐☐.☐kg Tick box with correct medication dose below.				
☐AL: Twice a day				
☐ Weight 5-14 kg: 1 tabs twice a da ☐ Weight 15-24 kg: 2 tabs twice a day ☐ Weight 25-34 kg: 3 tabs twice a day ☐ Weight >35 kg: 4 tabs twice a day				
Does subject have temperature > 38 ^{o}C? ☐ Yes ☐No If yes, give paracetamol now and two additional doses to take home.				
Antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Subject vomited?	Time of vomiting (hh:min)
Artemether–lumefantrine			☐ Yes ☐ No	
Other med(s) administered in study clinic (i.e. redose vomited antimalarial)				
Other medicines				

			☐ Yes ☐ No	
Medication given to go				
Name(s) of antimalarial drug(s)				Number of tablets
Artemether-lumefantrine				
Other meds (i.e. paracetamol): _____				
End-of-visit checklist				
☐Consent form completed (HO) ☐Day 0 CRF completed (HO) ☐Lab request form completed/reviewed (HO) ☐Symptom log completed (HO) ☐Enrolment log book completed (nurse)			☐Visit calendar completed (nurse) ☐Appointment card given (nurse) ☐Drug administered (nurse) ☐Travel reimbursement given (nurse)	

Study staff name: _____ Signature: _____

Case report form: day 1 (Form 5) Study nurse/HO		
Study ID_____ Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Place an additional subject identity number label in the lab log book.	Study arm: AL
Rule out severe malaria (HO)		
Presence of signs of severe or complicated malaria?☐ Yes ☐ No If YES, subject may have early treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 1 and send to lab. Complete Endpoint form, 4c. If NO, complete laboratory request form for day 1 and send to lab.		
Clinical/medication data (HO)		
Has subject had fever since the last visit? ☐ Yes ☐ No Temperature (axillary): ☐☐.☐ºC Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic? ☐Yes ☐ NoIf yes, list medications here: _____ How many blisters containing AL doses are empty? ☐ 0 ☐1 ☐ 2 (If 0, this is a protocol violation. Complete Endpoint form, 1a.) Did subject take the evening dose of AL? ☐Yes ☐No (If NO, this is a protocol violation. Complete Endpoint form, 1a.) If yes, did subject vomit within an hour after taking first dose? ☐ Yes ☐ No Did subject vomit within an hour after taking second dose? ☐Yes ☐ No ☐ N/A (If YES, this is involuntary study withdrawal. Complete Endpoint form, 4a.)		
Adverse events (HO)		
Did the subject have any problems caused by the medicine? ☐ Yes ☐ If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)?☐ Yes ☐ No, If yes, inform the study coordinator. Complete the symptom and adverse events (AE) log and record any new or worsening symptoms: _____ Send subject to nurse for medication administration.		
Medication administration (nurse)		
Subject weight:☐☐☐.☐kg Tick box with correct medication dose below.		

☐AL: Twice a day (Abamecha *et al.*)				
☐ Weight 5-14 kg: 1 tabs twice a day ☐ Weight 15-24 kg: 2 tabs twice a day			☐ Weight 25-34 kg: 3 tabs twice a day ☐ Weight >35 kg: 4 tabs twice a day	
Antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Subject vomited?	Time of vomiting (hh:min)
Artemether –lumefantrine			☐ Yes ☐ No	
Other med(s) administered in study clinic (i.e. redose vomited antimalarial) ☐ Yes ☐ No				
Medication given to go				
Name(s) of antimalarial drug(s)				Number of tablets
Artemether-lumefantrine				

End-of-visit checklist	
☐D*ay 1 CRF completed (nurse)*	☐*Visit log book comp*
☐*Lab request form completed/reviewed (MO)*	☐*Appointment card g*
☐*Symptom & AE log completed (HO)*	☐*Drug administered*
☐*Enrolment log book completed (nurse)*	☐*Travel reimburseme*
Other med(s) i.e. paracetamol: _____	

Study staff name: _____ Signature: _____

Case report form: day 2 (Form 6) Study nurse/HO		
Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Place an additional subject identity number label in the lab logbook.	Study arm: AL
Rule out severe malaria (HO)		
Presence of signs of severe or complicated malaria?☐ Yes ☐ No If YES, subject may have early treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 2 and send to lab. Complete Endpoint form (4c).If NO, complete laboratory request form for day 2 and send to lab.		
Clinical/medication data (HO)		
Has subject had fever since the last visit? ☐ Yes ☐ No Temperature (axillary): ☐☐.☐ ºC Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic?☐ Yes☐ No Ifyes, list medications here: _____ How many blisters containing AL doses are empty? ☐ 0 ☐1 ☐2 (If 0, this is a protocol violation. Complete Endpoint form, 1a.) Did subject take the evening dose of AL? ☐ Yes ☐ No (If NO, this is a protocol violation. Complete Endpoint form, 1a.) If yes, did subject vomit within an hour after taking first dose? ☐ Yes ☐ No Did subject vomit within an hour after taking second dose? ☐Yes ☐ No ☐ N/A (If YES, this is involuntary study withdrawal. Complete Endpoint form, 4a.)		
Adverse events (HO)		
Did the subject have any problems caused by the medicine? ☐ Yes ☐ No If yes, is it a serious problem(difficulty breathing, convulsions, and change in mental status)?☐Yes ☐No (If yes, inform the study coordinator.) Complete the symptom and adverse events (AE) logs and record any new or worsening symptoms:		
Parasite smear result review (HO)		
P. falciparum asexual parasites present? ☐ Yes ☐ No If yes, parasite density (parasites/ µL):☐☐☐☐☐ *P. falciparum* asexual parasite density (parasites/µL) on day 0: ☐☐☐☐☐		

Is current count higher than day 0 count? ☐ Yes ☐ No (If yes, subject has early treatment failure. Inform study coordinator and complete Endpoint form, 2a.)

P. falciparum gametocytes present? ☐ Yes ☐ No

If yes, gametocyte density (gametocytes/µL):☐☐☐☐

Other species present? ☐ Yes ☐ No (If yes, subject must be discharged from study. Complete Endpoint form, 14.)If yes, which species? ☐ *P. vivax* ☐ *P. ovali* ☐ *P. malariae*

Medication dispensing (nurse)

Patient weight: ☐☐☐.☐kg

Tick box with correct medication dose below.

☐**AL: Twice a day (Abamecha *et al.*)**

☐ Weight 5-14 kg: 1 tabs twice a day	☐ Weight 25-34 kg: 3 tabs twice a day
☐ Weight 15-24 kg: 2 tabs twice a day	☐ Weight >35 kg: 4 tabs twice a day

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the subject vomit?	Time of vomiting (hh:min)
Artemether – lumefantrine			☐ Yes ☐ No	
Other med(s) administered in study clinic (i.e. redose vomited antimalarial)				
			☐ Yes ☐ No	

Medication given to go

Name(s) of antimalarial drug(s)	Number of tablets
Artemether-lumefantrine	

End-of-visit checklist

☐Day 2 CRF completed (nurse/HO)	☐Visit log book completed (nurse)
☐Lab request form completed/reviewed (HO)	☐Appointment card given (nurse)
☐Symptom & AE logs completed (HO)	☐Drug administered (nurse)
☐Enrollment log book completed (nurse)	☐Travel reimbursement given (nurse)

.vivax ☐ P. oval ☐ P. malariae

Study staff name: _____ Signature: _____

Case report form: day 3 (Form 7) Study nurse/HO		
Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Subject identity number: Place an additional subject identity number label in the lab log book. Yellow	Study arm: AL

Rule out severe malaria (HO)

Presence of signs of severe or complicated malaria? ☐ Yes ☐ No

If YES, subject may have early treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 3 and send to lab. Complete Endpoint form, 4c. If NO, complete laboratory request form for day 3 and send to lab.)

Clinical/medication data (HO)

Has subject had fever since the last visit? ☐ Yes ☐ No Temperature (axillary): ☐☐.☐ºC

Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic?☐ Yes☐No If yes, list medications here: _____

How many blisters containing AL doses are empty? ☐ 0 ☐ 1 ☐ 2

(If 0, this is a protocol violation. Complete Endpoint form, 1a.)

Did subject take the evening dose of AL? ☐ Yes ☐ No (If NO, this is a protocol violation. Complete Endpoint form, 1a.)If yes, did subject vomit within an hour after taking first dose? ☐ Yes ☐ No

Did subject vomit within an hour after taking second dose? ☐ Yes ☐ No ☐ N/A

(If YES, this is involuntary study withdrawal. Complete Endpoint form, 4a.)

Adverse events (HO)

Did the subject have any problems caused by the medicine? ☐ Yes ☐No

If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)? ☐Yes ☐ No

(If yes, inform the study coordinator.) Complete the symptom and adverse events (AE) logs and record any new or worsening symptoms: _____

Lab result review (HO)

Copy results from lab below and follow prompts to the right.

Are *P. falciparum* asexual parasites present? ☐ Yes ☐ No If yes, early treatment failure

If yes, parasite density (parasites/µL):☐☐☐☐☐

Does subject have signs of severe malaria? ☐ Yes ☐No If yes, early treatment failure

Does subject have temperature > 37.5ºC or persistent fever? ☐ Yes ☐ No

P. falciparum asexual parasite density/µL on day 0: ☐☐☐☐☐

Is current count > 25% of day 0 count? ☐ Yes ☐ No If yes, early treatment failure

If subject has treatment failure:

☐inform study coordinator ☐complete Endpoint form (2a) ☐provide rescue treatment

Are *P. falciparum* gametocytes present? ☐ Yes ☐ No

If yes, gametocyte density (gametocytes/µL): ☐☐☐☐

Other species present? ☐ Yes ☐ No (If yes, complete Endpoint form, 14)

If yes, which species? ☐*P.vivax*☐*P. ovale* ☐*P. malariae*

End-of-visit checklist

☐D*ay 3 CRF completed (nurse)*	☐ *Visit log book completed (nurse)*
☐*Lab request form completed/reviewed (HO)*	*Appointment card given (nurse)*
☐*Symptom & AE logs completed (HO)*	☐*Travel reimbursement given (nurse)*
☐*Enrolment log book completed (nurse)*	

Study staff name: _____ Signature: _____

Case report form: day 7 (Form 8) Study nurse/HO		
Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Place an additional subject identity number label in the lab log book.	Study arm: AL
Rule out severe malaria (HO)		
Presence of signs of severe or complicated malaria? ☐ Yes ☐ No If YES, subject may have late treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 7 and send to lab. Complete Endpoint form 2b or 2c). If NO, complete laboratory request form for day 7 and send to lab.		
Clinical/medication data (HO)		
Has subject had fever since the last visit ☐ Yes ☐ No Temperature (axillary): ☐☐.☐ºC Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic?☐Yes ☐No If yes, list medications here: _____		
Adverse events (HO)		
Did the subject have any problems caused by the medicine? ☐ Yes ☐ No If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)?☐Yes ☐No (If yes, inform the study coordinator.) Complete the symptom and adverse events logs and record any new or worsening symptoms:		
Lab result review (HO)		
Copy results from lab below and follow prompts to the right. Are *P. falciparum*asexual parasites present?☐ Yes ☐ No If yes:parasite density (parasites/µL): ☐☐☐☐☐ Does subject have signs of severe malaria? ☐ Yes ☐ No does subject have temperature >37.5ºC or persistent fever? ☐ Yes ☐ No		← If yes, late parasitologic failure ← If yes OR ← Yes, late clinical failure.
If subject has treatment failure:☐ inform study coordinator ☐provide rescue treatment ☐ Endpoint form, 2b or 2c		

Are *P. falciparum* gametocytes present? ☐ Yes ☐No If yes, gametocyte density (gametocytes/µL):☐☐☐☐ Are species other than *P. falciparum* present? ☐ Yes☐ No (If yes, complete Endpoint form, 14) If yes, which species? ☐*P. ovale* ☐*P. malariae* ☐*P. vivax*	
End-of-visit checklist	
☐Day 7 CRF completed (nurse) ☐Lab request form completed/reviewed (HO) ☐Symptom & AE logs completed (HO)	☐Visit log book completed (nurse) ☐Appointment card completed (nurse) ☐Travel reimbursement given (nurse) ☐Enrolment log book completed (nurse)

Study staff name: _____ Signature: _____

Case report form: day 14 (Form 9)	**Study nurse/HO**	
Study ID_____ Date of visit:_____/_____/_____GC (day / month / year)	subject ID number label *Place an additional subject identity number label in the lab log book.*	Study arm: AL

Rule out severe malaria (HO)

Presence of signs of severe or complicated malaria ?☐Yes ☐No **If YES,** subject may have late treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab requestform for day 14 and send to lab. Complete Endpoint form (2 b or 2c).

If NO, complete laboratory request form for day 14 and send to lab.

Clinical/medication data (HO)

Has subject had fever since the last visit? ☐Yes ☐No Axilary temperature (°C): ☐☐.☐

Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic?☐Yes☐NoIf yes, list medications here: _____

Adverse events (HO)

Did the subject have any problems caused by the medicine? ☐ Yes ☐No

If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)? ☐Yes ☐No

(If yes, inform the study coordinator.) ***Complete the symptom and adverse events logs and record any new or worsening symptoms:***

Lab result review (HO)

Hemoglobin on day 0:☐☐.☐g/dL

Copy results from lab below and follow prompts to the right.

Are *P. falciparum* asexual parasites present? ☐Yes ☐NoIf yes:

- parasite density (parasites/µL): ☐☐☐☐☐☐
- Does subject have signs of severe malaria? ☐Yes ☐No
- Does subject have temperature >37.5 ºC or persistent fever? ☐Yes ☐No

☐ **If yes, late parasitologic failure**
☐ **If yes OR** ☐ **yes, Late clinical failure.**

If subject has treatment failure:

☐ **inform study coordinator** ☐**provide rescue treatment** **Endpoint form, 2b or 2c**

Are *P. falciparum (circle one)* gametocytes present? ☐Yes ☐No

If yes, gametocyte density (gametocytes/µL): ☐☐☐☐☐☐

Are species other than *P. falciparum* present? ☐Yes ☐No **(If yes, complete Endpoint form, 14.)**

If yes, which species? ☐*P.vivax* ☐*P. ovale* ☐*P. malariae*

If endpoint not reached, send to study dispensary for medication administration.

End-of-visit checklist

☐ Day 14 CRF completed (nurse)	☐ Visit log book completed (nurse)
☐ Lab request form completed/reviewed (HO)	☐ Appointment card completed (nurse)
☐ Symptom & AE logs completed (HO)	☐ Travel reimbursement given (nurse)
☐ Enrolment log book completed (nurse)	

Study staff name: _____ Signature: _____

Case report form: day 21 (Form 11)	**Study nurse/HO**	
Study ID_____ Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Place an additional subject identity number label in the lab log book. Subject ID	Study arm: AL

Rule out severe malaria (HO)

Presence of signs of severe or complicated malaria? ☐ Yes☐ No

If YES, subject may have late treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 21 and send to lab. Complete Endpoint form (2 b or 2c).

If NO, complete laboratory request form for day 21 and send to lab.

Clinical/medication data (HO)

Has subject had fever since the last visit? ☐ Yes ☐ No Temperature (axillary): ☐☐.☐ºC

Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic?☐ Yes ☐ No

If yes, list medications here: _____

Adverse events (HO)

Did the subject have any problems caused by the medicine? ☐ Yes ☐ No

If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)?

☐ Yes ☐No

(If yes, inform the study coordinator.) Complete the symptom and adverse events (AE) logs and record any new or worsening symptoms:

Lab result review (HO)

Copy results from lab below and follow prompts to the right. Are *P. falciparum* asexual parasites present? ☐ Yes ☐No If yes: parasite density (parasites/µL): ☐☐☐☐☐ Does subject have signs of severe malaria? ☐ Yes☐ No does subject have temperature > 37.5ºC or persistent fever? ☐ Yes ☐No	← If yes, late parasitologic failure ← If yes OR ← yes, late clinical failure.

If subject has treatment failure:

☐ inform study coordinator ☐provide rescue treatment ☐ Endpoint form, 2b or 2c	
Are *P*. falciparum gametocytes present?(circle one) ☐Yes ☐ No If yes, gametocyte density (gametocytes/µL): ☐☐☐☐ Are species other than *P. falciparum* present? ☐ Yes ☐No (If yes, complete Endpoint form, 14.) If yes, which species? ☐P.vivax ☐ P. ovale ☐P. malariae	
End-of-visit checklist	
☐Day 21 CRF completed (nurse) ☐Lab request form completed/reviewed (HO) ☐Symptom & AE logs completed (HO) ☐Enrolment log book completed (nurse)	☐Visit log book completed (nurse) ☐Appointment card completed (nurse) ☐Travel reimbursement given (nurse)

Study staff name: _____ Signature: _____

Case report form: day 28 (Form 12) **Study nurse/HO**		
Study ID_____ Date of visit: __ __ / __ __ / __ __ __GC (day / month / year)	Subject identity number: Place an additional subject identity number label in the lab log book. Subject ID number label	Study arm: AL
Rule out severe malaria (HO)		
Presence of signs of severe or complicated malaria (see Annex III)? ☐ Yes ☐ No If YES, subject may have late treatment failure. Discuss with study coordinator, arrange for hospital transfer and pre-transfer treatment. If it will not delay care, complete lab request form for day 28 and send to lab. Complete Endpoint form, 2 b or 2c. If NO, complete laboratory request form for day 28 and send to lab.		
Clinical/medication data (HO)		
Has the subject had fever since the last visit?☐ Yes ☐ No Temperature (axillary): ☐☐.☐ºC Since subject's last visit to this clinic, have they taken any medication not prescribed by this clinic? ☐ Yes ☐No If yes,list medications here: _____		
Adverse events (HO)		
Has the subject been having any problems with their health? ☐ Yes ☐ No If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)?☐ Yes☐ No (If yes, inform the study coordinator.) Complete the symptom and adverse events logs and record any new or worsening symptoms		
Lab result review (HO)		
Hemoglobin:☐☐ .☐g/dl		
Copy results from lab below and follow prompts to the right. Are *P. falciparum*asexual parasites present? (circle one)☐ Yes ☐ No If yes: parasite density (parasites/µL):☐☐☐☐☐ Does subject have signs of severe malaria? ☐ Yes ☐ No does subject have temperature >37.5ºC or Persistent fever? ☐Yes ☐No	← If yes, late parasitological failure ← If yes OR ← yes, Late clinical failure.	

If subject has treatment failure ☐ inform study coordinator ☐ provide rescue treatment ☐ Endpoint form, 2b or 2c	
Are *P. falciparum* gametocytes present? ☐ Yes ☐No If yes, gametocyte density (gametocytes/µL): ☐☐☐☐ Are species other than *P. falciparum* present? ☐ Yes ☐No (If yes, complete Endpoint form, 14) If yes, which species? ☐P.vivax ☐P. ovale ☐P. malariae	
End-of-visit checklist	
☐Day 28 CRF completed (nurse) ☐Lab request form completed/reviewed (HO) ☐Symptom & AE logs completed (HO) ☐Enrolment log book completed (nurse)	☐Visit log book completed (nurse) ☐Appointment card completed (nurse) ☐Travel reimbursement given (nurse)

Study staff name: _____ Signature: _____

Case report form: unscheduled visit (Form 13) **Study nurse/HO**		
Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)	Subject identity number: *Place an additional subject identity number label in the lab log book.*	Study arm: AL
Rule out severe malaria (HO)		
Presence of signs of severe or complicated malaria ?☐ Yes ☐ No **If YES, subject may have treatment failure or malaria recurrence. Discuss with study coordinator, arrange for hospital transfer, complete lab request form for unscheduled visit, send to lab, and complete Endpoint form.** **If NO, complete laboratory request form for unscheduled visit and send to lab.**		
Clinical/medication data (HO)		
Has subject had fever in past 24 hours ? ☐Yes ☐No Temperature (axillary):☐☐.☐ºC Since subject's visit to this clinic, have they taken any medication not prescribed by this clinic? ☐ Yes ☐ No If yes, list medications here: _____		
Adverse events (HO)		
Did the subject have any problems caused by the medicine? ☐ Yes ☐ No If yes, is it a serious problem (difficulty breathing, convulsions, and change in mental status)? ☐ Yes ☐ No **(If yes, inform the study coordinator.)** ***Complete the symptom and adverse events logs and record any new or worsening symptoms:*** **_____**		
Lab result review (HO)		
Haemoglobin: ☐☐.☐ g/dl Haemoglobin on day 0:☐☐.☐ g/dl ***Copy results from lab below and follow prompts to the right.*** Are *P. falciparum (circle one)* asexual parasites present? ☐ Yes ☐ No If yes: parasite density (parasites/µL):☐☐☐☐☐ Does subject have signs of severe malaria? ☐ Yes ☐ No does subject have temperature > 37.5ºC or persistent fever?☐ Yes ☐ No **If subject has treatment failure: ☐ inform study coordinator ☐ provide rescue treatment ☐complete Endpoint form** Are *P. falciparum)* gametocytes present? ☐ Yes ☐No If yes, gametocyte density (gametocytes/µL):☐☐☐☐☐ Are species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, complete Endpoint form,*14*)** If yes, which species? ☐*P.vivax* ☐*P. ovale* ☐*P. malariae* If subject does not have parasitemia and has symptoms or fever, refer to seek further care and write to where referred here:		
End-of-visit checklist		
☐*Unscheduled visit CRF completed (nurse)* ☐*Lab request form completed/reviewed (HO)* ☐*Symptom & AE logs completed (HO)*	☐*Enrolment log book completed (nurse)* ☐*Appointment card given back—no new appt (nurse)* ☐*Travel reimbursement given (nurse)*	

Study staff name: _____ Signature: _____

Annex 9: Endpoint form

<table>
<tr><td colspan="3">Endpoint form (Form 14) HO/nurse</td></tr>
<tr><td>Study ID_____
Date of visit: __ __ / __ __ / __ __ __ __GC
(day / month / year)</td><td>Place an additional subject identity number label in the lab log book.
Subject ID number label</td><td>Study arm: AL</td></tr>
<tr><td colspan="3">Endpoint classification (HO/study supervisor)</td></tr>
<tr><td colspan="3">☐1. Protocol violation: choose one from options below
☐ 1a. Failure to complete treatment
☐1b. Erroneous inclusion of a patient outside of the inclusion/exclusion criteria
☐1c. Misclassification of a patient due to a laboratory error leading to administration of rescue treatmen
☐1d. patients taking medication with antimalarial activity during study period without being directed by the study clinicians</td></tr>
<tr><td colspan="3">☐2. Treatment failure (classify below, and write parasite count if available:☐☐☐☐☐parasites/µL)</td></tr>
<tr><td>Day 1, 2, or 3</td><td colspan="2">☐ 2a. Early treatment failure (ETF):
☐ Danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitemia
☐Parasitemia on day 2 higher than on day 0
☐Parasitemia on day 3 with axillary temperature ≥ 37.5 ºC
☐Parasitemia on day 3 ≥ 25% of count on day 0</td></tr>
<tr><td>Day 4-42</td><td colspan="2">☐2b. Late clinical failure (LCF):
☐No previous early treatment failure AND
☐Danger signs or severe malaria in the presence of parasitemia OR
☐Parasitemia AND ☐ Axillary temperature ≥ 37.5 ºC or history of fever</td></tr>
<tr><td>Day 7-42</td><td colspan="2">☐2c. Late parasitological failure:
☐No previous early treatment failure or late clinical failure AND
☐Parasitemia AND ☐ Axillary temperature < 37.5 ºC</td></tr>
</table>

☐3. Withdrawal from study (voluntary)—i.e., withdrawal of consent	
☐4. Withdrawal from study (involuntary): ☐4a. Vomiting both initial/replacement doses ☐4b. Severe side effects necessitating hospitalization or discontinuation of medication ☐4c. Progression to severe malaria	
☐5. Loss to follow-up	
☐6. Occurrence during follow-up of concomitant illness that interferes with classification of outcome (including mixed infection and other Plasmodium species)	
☐7. Completion of follow-up period without treatment failure	
Decision-making	
Does patient have signs of severe or complicated malaria? ☐Yes ☐ No If yes, patient may need referral for hospital admission. Discuss with study coordinator, consider oral quinine or IV artesunate, and describe decision-making and course in Adverse Event Log. Does patient have other species of malaria symptoms without parasitemia? ☐Yes ☐ No If yes, discharge from study; refer to health care facility for treatment per national guidelines. If patient has treatment failure, go to "rescue therapy" on reverse. If patient didn't complete the treatment, record as a protocol violation or withdrawal of consent, give full course of study arm medication	
Rescue therapy (HO/SS)	Study arm medication (HO/SS)
Subject weight: ☐☐☐. ☐ kg	
Reason for rescue therapy: ☐ Early treatment failure ☐ Late clinical failure ☐ Late parasitological failure Calculate rescue therapy dose of oral quinine: subject weight ↓ ☐☐☐.☐kg X 10 mg/kg =☐☐☐.☐ mg	Is patient pregnant? ☐ Yes ☐ No If yes, discharge from follow up & treat according to guideline. If no, choose appropriate dose of study arm meds: ☐AL: Twice a day (Abamecha *et al.*) ☐ Weight 5-14 kg: 1 tabs twice a day ☐ Weight 15-24 kg: 2 tabs twice a day

every 8 hours for 7 days Dose in mg / 300 = number of pills to give = _____ pills Maximum adult dose = 600 mg every 8 hours for 7 days.	☐ Weight 25-34 kg: 3 tabs twice a day ☐ Weight >35 kg: 4 tabs twice a day
Send patient to dispensary for drug administration. Directly observed therapy is not necessary.	
Medication administration (nurse)	
Antimalarial drug(s)	Number of tablets
Artemether – lumefantrine	
Other med(s) administered in study clinic (i.e. paracetamol):	
Medication given to go	
Name(s) of antimalarial drug(s)	Number of tablets
Artemether – lumefantrine	
Follow-up (nurse)	
If patient received rescue treatment, make follow up appointment for next day.	
End-of-visit checklist	
☐Endpoint visit CRF completed (HO/nurse) ☐Lab request form completed/reviewed (HO) ☐Enrolment log book completed (nurse) ☐Visit log completed (nurse)	☐Appointment card given (nurse) ☐Drug administered (nurse) ☐Travel reimbursement given (nurse)

Study staff name: _____ Signature: _____

Annex 10: Effectiveness study: adverse event log

Effectiveness Study: Adverse Event log								**HO/Nurse**
Study ID_____ Date of visit: __ __ / __ __ / __ __ __ __GC (day / month / year)		Subject ID number label *Place an additional subject identity number label in the lab log book.*				**Study arm: AL**		
No	Adverse Event (AE)	Date AE started	S	DR	SAE	Date AE ended	O	Actions taken
1		__ __/__ __/__ __ Ini tials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	
2		__ __/__ __/__ __ initials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	
3		__ __/__ __/__ __ initials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	
4		__ __/__ __/__ __ initials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	
5		__ __/__ __/__ __ initials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	
6		__ __/__ __/__ __ initials_____	___	☐Yes ☐ No	☐Yes ☐ No	__ __/__ __/__ __ initials_____	___	

Severity: 1= Mild - Transient or mild discomfort (<48 hours); no intervention required.

2=Moderate - Mild to moderate limitation of activity-some assistance may be needed; no or minimal medical intervention/therapy required.

3=Severe - Marked limitation in activity, some assistance usually required; medical intervention/therapy required; hospitalization possible.

4= Life threatening

Drug Related (DR): Yes= May be related to the study medication, No=Not related to the study medication

Outcome (O):1 =Resolved, 2= Resolved with sequellae, 3=Ongoing, 4 =Deceased 0=Unknown

SAE: Yes=SAE form completed by supervisor/HO and submitted to principal investigator, FMHACA and study IRBs, No=No SAE form needed

Study staff name: _____ Signature: _____

Annex 11. Effectiveness study: symptoms log

Effectiveness Study: Symptoms Log (Score '0' if symptom is absent, or '1' if symptom is present) **HO/ nurse**									
Study ID_____ Date of visit: _____/ _____/ _____GC (day / month / year)				Subject ID Number label *Place an additional subject identity number label in the lab log book.*					Study arm: AL
Follow-up day	0	1	2	3	7	14	21	28	Unscheduled visit
Fevers									
Chills									
Headache									
Nausea									
Vomiting									
Abdominal Pain									
Diarrhea									
Cough									
Weakness									
Body ache									
Itching									
Rash									
Eye discharge/lesions									
Mouth lesion									
Dark urine									
others									

Study staff name: _____ Signature: _____

Annex 12: Consent form for adult (≥18 years):

Title of the project: therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated plasmodium falciparum in Asayita district, northeast Ethiopia

Name of principal investigator: _____

Address: _____

Your doctor has concluded that you have *Plasmodium falciparum*-induced malaria and that you require therapy with a drug called Artemether-lumefantrine. The FMOH presently recommends Artemether-lumefantrine as the first line medication for the treatment of uncomplicated *P. falciparum* malaria in Ethiopia. This medical facility will give you free Artemether-lumefantrine medication for your malaria illness regardless of whether you choose to take part in the study.

If you could volunteer for this research project, which will enable us to closely monitor and record the progression of your malaria infection following treatment with AL, we would greatly appreciate your assistance. The AHRI carries out TES on a regular basis to verify the continued effectiveness of approved malaria therapies. Asayita is one of the six sites chosen for this study, where we plan to evaluate the effectiveness of Artemether-lumefantrine in treating malaria. The results of this study should assist administrators of national malaria control programs in assessing whether there is evidence of drug resistance to Artemether-lumefantrine and whether alternative drugs would be needed in place of Artemether-lumefantrine.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study.

If you agree, you will be treated with six doses of Artemether-lumefantrine given twice daily for 3 days. (This is the same treatment that you would receive if you decide not to volunteer for this study.) The first dose will be given at the clinic supervised by study nurse and the 2nd dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days 1, 2, 3, 7, 14, 21 and 28. You will also be asked to come to the clinic at any other time if you become sicker, develop new symptoms, or if you fail to get better. Transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain a finger prick blood samples from you by a qualified technician that would be used only for malaria diagnosis, to detect the presence of markers for malaria drug resistance, and to see the outcome of treatment. There is no

serious risk in participating, but you may experience a small pain during finger pricking. The pain should disappear within 1 day.

The Artemether-lumefantrine medicine can have some unwanted side-effects or some effects that we are not currently aware of; however, we will follow you closely and ensure proper medical treatment. If you take Artemether-lumefantrine as directed, the course of your illness and possible side effects from Artemether-lumefantrine should not be any different whether you volunteer for this study or not. The Artemether-lumefantrine medicine may have some unexpected effects; however, we will follow you closely and keep track of these effects, if they arise. Patients showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throughout the study period.

Your participation in this study is completely voluntary and you can refuse to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services you receive at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive at the clinic will continue. If you decide to participate in this study, the information in your records is strictly confidential and your name will not be used in any report and any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Do you understand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

I have been invited to participate in Artemether-lumefantrine effectiveness study. I have read the information in this consent form or have been readout to me in my own language. I clearly understand the content. I am also aware of my right to optout of the study at any time during the course of the study without having to give reasons for doing so. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction and I voluntarily consent to participate in the study

Name of participant: _____

Signature of participant: _____

Date: _____

(dd/mm/yyyy)

Witness' signature: I confirm that the participant has given consent freely.

Print name of witness: _____

Signature of witness: _____

Date: _____

(dd/mm/yyyy)

Investigator's signature:

I confirm that the participant has given consent freely. Print name of investigator:_____

Signature of investigator: _____

Date: _____

(dd/mm/yyyy)

ለአዋቂ የተሰናዳ የስምምነት ቅጽ

ሀ/ ጥናቱን የተመከቱ መረጃዎች

1. የጥናቱ ርዕስ

የፐላዝሞዲየም ፋልሲፓረም ህክምና ጥቅም ላይ ሲውሌ 19 ዓመታት ያስቆጠረውን የኳርተም መድሐኒት ፍቱንነት ማጥናት፡ ፡ (Therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated *P. falciparum* in Asayita district, northeast Ethiopia).

ጥናቱን የሚያጠናው ተመራመሪ ስም፡- ----------

አድራሻ፡- _____

2. **<u>የጥናቱ ዓላ</u>**፡-

ኳርተም የወባ መድሒኒት የፐላዝሞዲየም ፋልሲፓረም ወባ ህክምና የሚሰጥ የመጀመሪያ ደረጃ መድሐኒት ነው፡፡ የፌደራል ጤና ጥበቃ ሚኒስቴር የወባ መድሐኒቶችን ፍቱንነት በየጊዜው በሚደረጉ ጥናቶች ይፈትሻል፡፡ ይህ ጥናትም የዚህ አይነቱ ፍተሻ አካል ሲሆን በአርማዎር ሀንሰን የምርምር ተቋም የሚካሄዴ የካርተም መዴሐኒት ፍቱነንት ፍተሻ ጥናት ነው፡፡

ይህ ጥናት በ6 የኢትዮጵያ አካባቢዎች ወባን በመፈወስ ረገድ ኳርተም ምን ያህል እየሰራ እንደሆነ በመገምገም መድሐኒቱ አሁንም ድረስ ፍቱን ሆኖ መዝለቁንና አለመዝለቁን ማረጋገጥ የሚደረግ ሲሆን ከጥናቱ የሚገኘው መረጃ ወባው መድሐኒቱን መላመድና አለመላመዱን መለየት ብሎም የመድሐኒቱ ምትክ ማስፈለጉንና አለማስፈለጉን ለመወሰን ጥቅም ላይ ይውላል፡፡ የጥናቱ ዓብይ ዓላማም ይሄው ነው፡፡

3. **<u>በጥናቱ የሚታቀፉ ታካሚዎች</u>**

ይህን ጥናት ለማጥናት እድሜያቸው 6 ወር እና ከዛ በላይ የሆናቸው የወባ ታማሚዎች በበጎ ፈቃደኝነት ተሳታፊ ይሆናለ፡፡ ከዚህ አኳያ እርስዎ በኳርተም መድሐኒት በሚታከም ወባ ሳቢያ መታመምዎ በምርመራ ከተረጋገጠ በጥናቱ እንዲካተቱና ለጥናቱ እገዛ እንዲያዯርጉ የእርስዎ ፈቃድ ያስፈልጋል፡፡

4. **<u>እርስዎ ፈቃድዎን ገልጸው በጥናቱ ከታቀፉ በጥናቱ የሚሳተፉት እንዴት ነው?</u>**

በጥናቱ ላይ ለመሳተፍ ከተስማሙ፣ በቀን ሁለት ጊዜ ለ 3 ቀናት በሚሰጥ 6 የ Artemether-lumefantrine መጠን ይታከማሉ። (ለዚህ ጥናት በበጎ ፈቃደኝነት ላለመሳተፍ ከወሰኑ እርስዎ የሚወስዱት ተመሳሳይ ህክምና ነው።) የመጀመሪያው ልክ መጠን በጥናቱ ላይ በሚሳተፍ የህክምና ባለሙያ ክሊኒክ የሚሰጥ ሲሆን በቀን 2 ጊዜ ለ3 ቀናት የሚሰጥ ሲሆን በቀጣዩ ቀናት ጧት ጧት ወደ ጤና ጣቢያው እየመጡ መድሐኒቱን ይወስዱና ማታ የሚዋጠውን ደግሞ ተቀብል ወደ ቤቶ ይመለሳሉ፡ ፡ ጥናቱ በ28 ቀናት ውስጥ ይካሄዳል። በዛን ጊዜ 1, 2, 3, 7, 14, 21 እና 28 ወደ ጤና ተቋም እንዲመጡ ይጠየቃሉ. በተጨማሪም በማንኛውም ጊዜ ከታመሙ ወደ ክሊኒኩ እንዲመጡ ይጠየቃሉ፡፡ መድሐኒቱን ወስደው ሳይሻልዎት ቢቀርና አዲስ የወባ በሽታ ምልክት ቢያሳዩ ከመርሀግብሩ በተጨማሪ በማናቸውም ወቅት ወደ ጤና ጣቢያው እንዲመጡ ተደርጎ ከትትል እየተደረገልዎት ይታከማለ፡፡

5. **<u>በጥናቱ ወቅት ለእርስዎ የሚደረጉት የወባ ምርመራዎች</u>**

በጥናቱ ውስጥ ህክምናውን በሚከታተሉበት ወቅት ከጣት ላይ ደም እየተወሰደ የወባ ምርመራ ይደረግልዎታል፡፡ ከጣት ደም ሲወሰድ ጣት ስለሚበጣ ጥቂት ለአጭር ጊዜ ብቻ የሚቆይ የህመም ስሜት ይኖረዋል፡፡ በተወሰደው ደም የሚደረገው ምርመራ እየተሻለዎ መምጣቱን እንዲሁም ወባው ለመድሐኒቱ ያለውን ብግርነት ያሳያል፡፡

6. ለእርስዎ የሚሰጠው የኮአርተም መዴሐኒት

የኳርተም መድሐኒት በውል ተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታሌ፡፡ ሆኖም ይህ ከጥናቱ ጋር የሚያገናኘው አንዳች ነገር የለም፡፡ የመዴሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻልው እየባሰበት የሚሄዴ ታካሚ ከተገኘ በወባ የህክምና መመሪያ መሰረት በተሻለ መድሐኒት እንዲታከም ይደረጋል፡፡

7. <u>በጥናቱ በመሳተፍዎት ያልዎት መብትና ጥቅም</u>

በዚህ ጥናት ውስጥ መካተት የሚቻለው በፈቃደኝነት ብቻ ስለሆነ በጥናቱ ያለመሳተፍ ሙለ መብት አልዎት፡፡ በጥናቱ ውስጥ አልሳተፍም በማለትዎ የሚያጡት እንከብካቤም ሆነ ህክምና አይኖርም፡፡ በጥናቱ ውስጥ መሳተፍ ከጀመሩም በኋላም ቢሆን እንኳ ከጥናቱ መውጣት ቢፈሌጉ ሙሉ መብት ያልዎት በመሆኑ የሚያስፈልገውን ህክምና በሙለ አንዴም ሳይጎልብዎት ከጤና ጣቢያው ያገኛለ፡፡

በሌላ በኩል በዚህ ጥናት ውስጥ የሚታቀፍ ማንኛውም ታካሚ በተሰናዳው መርሃ ግብር መሰረት ወደ ጤና ተቋሙ ሲመላለስ ለእያንዳንዱ ቀን የመሄጃና የመምጫ የመጓጓጉ አበል ይሰጠዋል፡፡ በተጨማሪም ጥናቱ ከታካሚው የሚያገኘውን መረጃ ፍጹም ምስጢራዊነት የሚጠብቅ ሲሆን ከጥናቱ ጋር ተያይዞ በሚወጣ ማንኛውም ዓይነት ሪፖርት ውስጥ የታካሚው ስም አይጠቀስም፡ :

ከላይ የተዘረዘሩትን ጥናቱን የተመለከቱ መረጃዎች በሚገባ መረዳትዎን ያረጋግጡ፡፡ ያልገባዎት አረፍተ ነገር ወይም ግልጽ ያልሆነ ሃሰብ ካለም ማንኛውንም ማብራሪያ መጠየቅ ይችላሉ፡፡ ሁሉም መረጃዎች በሚገባ ግልጽ ከሆነልዎትና በጥናቱ እንዲታቀፉ የሚፈቅዱ ከሆነ ከስር የተቀመጠውን የስምምነት ሰነድ ተረድተው ይፈርሙ፡፡

ለ/ **<u>የስምምነት ሰነድ</u>**

ጥናቱን የተመለከተውን መረጃ አንብቤ/በቋንቋየ ተነቦልኝ ይዘቱን በውል ተገንዝቤአለሁ፡፡ አስፈሊጊ ሆኖ ካገኘሁት በፈለኩት በማናኛውም ጊዜ ጥናቱን የማቋረጥ መብት እንዲለኝ በሚገባ አውቄአለሁ፡፡ መረጃውን ካነበብኩ/ከተነበበልኝ በኋሊ ያልገባኝን ነገር ጠይቄና ተረድቼ በጥናቱ እንድታቀፍ በፈቃደኝነት ተስማምቻለሁ፡፡

የጥናቱ ተሳታፊ ስም _____ፊርማ _____ ቀን ----------

የምስክር ስም _____ፊርማ _____ቀን ----------

የጥናት አድራጊው ስም _____ፊርማ _____ ቀን ----------

Annex 12: Consent form for children

Title of the project: therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated plasmodium falciparum in Asayita district, Northeast Ethiopia

Name of principal investigator: _____

Address: _____

Your health care provider has determined that your child has malaria infection caused by *Plasmodium falciparum* that needs treatment with a medication called Artemether-lumefantrine. Artemether-lumefantrine is currently the recommended 1st line drug by the FMOH for the treatment of uncomplicated *Plasmodium falciparum* malaria in Ethiopia. Regardless of whether you decide to allow your child to participate in this study, this health care facility will provide your child with Artemether-lumefantrine medication for this malaria infection at no cost to you. We would appreciate your help if you decide to allow your child to volunteer for this research study that will help us to carefully follow and document the course of this malaria infection after treatment with Artemether-lumefantrine. The Ministry regularly conducts clinical studies to make sure that the recommended malaria treatments are still working well. In this study we intend to assess how well Artemether-lumefantrine works to cure malaria at six locations in Ethiopia, and to determine whether it is still working as well as in our earlier studies. The information from this study should help national malaria control program managers to determine whether there is evidence of Artemether-lumefantrine drug resistance and whether we may need to find other medications to substitute for Artemether-lumefantrine.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study.Thus, you are being asked to consent to have your child participate in this study. If you agree, your child will be treated with six doses of Artemether-lumefantrine given twice daily for 3 days. (This is the same treatment that your child would receive if you decide not to volunteer your child for this study.) The first dose will be given at the clinic supervised by study nurse and the 2nd dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days 1, 2, 3, 7, 14, 21 and 28. You will also be asked to bring your child back to the clinic at any other time if your child becomes sicker, develops new symptoms, or if your child fails to get better Transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain a finger prick blood samples from your child by a qualified technician that

would be used only for malaria diagnoses, to detect the presence of markers for drug resistance, and to see the outcome of treatment. There is no serious risk in participating but your child may experience a small pain during finger pricking. The pain should disappear within 1 day. The Artemether-lumefantrine medicine can have some unwanted side-effects or some effects that we are not currently aware of; however, but we will follow your child closely and ensure proper medical treatment. If your child takes the Artemether-lumefantrine as directed, the course of your child's illness and possible side effects from Artemether-lumefantrine should not be any different whether you volunteer your child for this study or not. Children showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throughout the study period.

Child to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services your child receives at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services your child receives at the clinic will continue.

If you decide that your child will participate in this study, the information in your child records is strictly confidential and your child name will not be used in any report and any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Do you understand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of consent

I have been invited to have my child participate in a coartem effectiveness study. I have read the information in this consent form or have been readout to me in my own language. I clearly understand the content. I am also aware of my right to out of the study at any time during the course of the study without having to give reasons for doing so. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction and I voluntarily consent to my child's participation in this study.

Parent name of participant: _____

Parent name of caregiver: _____

Signature of caregiver: _____

Date: _____

(dd/mmm/yyyy)

Witness' signature: I confirm that the child's caregiver has given consent freely.

Parent name of witness: _____

Signature of witness: _____

Date: _____

(dd/mmm/yyyy)

Investigator's signature:

I confirm that the caregiver has given consent freely. Print name of investigator: _____

Signature of investigator: _____

Date: _____

(dd/mmm/yyyy)

<u>ለህፃናት የተሰናዳ የስምምነት ቅጽ</u>

ሀ/ <u>ጥናቱን የተመለከቱ መረጃዎች</u>

1. **<u>የጥናቱ ርዕስ</u>**

   የፐላዝሞዲየም ፋልሲፓረም ህክምና ጥቅም ላይ ሲውሌ 19 ዓመታት ያስቆጠረውን የኳርተም መድሐኒት ፍቱንነት ማጥናት፡፡ (Therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated *plasmodium falciparum* in Asayita district, northeast Ethiopia).

   ኳርተም የወባ መድሒነት የፐላዝሞዲየም ፋልሲፓረም ወባ ህክምና የሚሰጥ የመጀመሪያ ደረጃ መድሐኒት ነው፡፡ የፌደራል ጤና ጥበቃ ሚኒስቴር የወባ መድሐኒቶችን ፍቱንነት በየጊዜው በሚደረጉ ጥናቶች ይፈትሻል፡፡ ይህ ጥናትም የዚህ አይነቱ ፍተሻ አካል ሲሆን በአርማዎር ሀንሰን የምርምር ተቋም የሚካሄዴ የካርተም መዴሐኒት ፍቱነንት ፍተሻ ጥናት ነው፡፡

2. **<u>የጥናቱ ዓላማ</u>**

   ይህ ጥናት በ6 የኢትዮጵያ አካባቢዎች ወባን በመፈወስ ረድ ኳርተም ምን ያህል እየሰራ እንደሆነ በመገምገም መድሐኒቱ አሁንም ዴ,ድረስ ፍቱን ሆኖ መዝለቁንና አለመዝለቁን ማረጋገጥ የሚደረግ ሲሆን ከጥናቱ የሚገኘው መረጃ ወባው መድሐኒቱን መላመድና አለመላመዱን መለየት ብልም የመድሐኒቱ ምትክ ማስፈለጉንና አለማስፈለጉን ለመወሰን ጥቅም ላይ ይውላል፡፡ የጥናቱ ዓብይ ዓላማም ይሄው ነው፡፡

3. **<u>በጥናቱ የሚታቀፉ ታካሚዎች</u>**

   ይህን ጥናት ለማጥናት እድሜያቸው 6 ወር እና ከዛ በላይ የሆናቸው የወባ ታማሚዎች በበጎ ፈቃደኝነት ተሳታፊ ይሆናሉ፡፡ ከዚህ አኳያ ልጅዎ በኳርተም መድሐኒት በሚታከም ወባ ሳቢያ መታመሙ በምርመራ ከተረጋገጠ በጥናቱ እንዲካተትና ለጥናቱ እገዛ እንዲያደርግ የእርሰዎ ፈቃድ ያስፈልጋል፡፡

4. **<u>እርሰዎ ፈቃድዎን ገልጸው ልጅዎ በጥናቱ ከታቀፈ በጥናቱ የሚሳተፈው እንዴት ነው?</u>**

   በጥናቱ ላይ ለመሳተፍ ከተስማሙ፣ በቀን ሁለት ጊዜ ለ 3 ቀናት በሚሰጥ 6 የ Artemether-lumefantrine መጠን ያልዉ መድሐኒት ይታከማል። (ለዚህ ጥናት በበጎ ፈቃደኝነት ልጅዎን ላለማሳተፍ ከወሰኑ ለልጅዎ የሚሰጠው ተመሳሳይ ህክምና ነው።) የመጀመሪያው ልክ መጠን በጥናቱ ላይ በሚሳተፍ የህክምና ባለሙያ ክሊኒክ የሚሰጥ ሲሆን በቀን 2 ጊዜ ለ3 ቀናት የሚሰጥ ሲሆን ልጅዎ በቀጣዩ ቀናት ጧት ጧት ወደ ጤና ጣቢያው እየመጡ መድሐኒቱን ይወስዱና ማታ የሚዋጠውን ደግሞ ተቀብል ወደ ቤቶ ይመለሳል፡፡ በዛን ጊዜ 1, 2, 3, 7, 14, 21 እና 28 ወደ ጤና ተቋም እንዲመጣ ይጠየቃሉ፡፡ በተጨማሪም በማንኛውም ጊዜ ከታመመ ወደ ክሊኒኩ እንዲመጣ ይደረጋል፡፡ መድሐኒቱን ወስዶ ሳይሻለው ቢቀርና አዲስ የወባ በሽታ ምልክት ቢያሳይ ከመርሀግብሩ በተጨማሪ በማናቸውም ወቅት ወደ ጤና ጣቢያው እንዲመጣ ተደርጎ ክትትል እየተደረገለት ይታከማል፡፡

5. **<u>በጥናቱ ወቅት ለልጅዎ የሚደረጉት የወባ ምርመራዎች</u>**

   ልጁ በጥናቱ ውስጥ ህክምናውን በሚከታተልበት ወቅት ከጣት ላይ ደም እየተወሰደ የወባ ምርመራ ይደረግለታል፡፡ ከጣት ደም ሲወሰድ ጣት ስለሚበጣ ጥቂት ለአጭር ጊዜ ብቻ የሚቆይ የህመም ስሜት ይኖረዋል፡፡ በተወሰደው ደም የሚደረገው ምርመራ ልጅዎ እየተሻለው መምጣቱን እንዲሁም ወባው ለመድሐኒቱ ያለውን ብግርነት ያሳያል፡፡

6. **<u>ለልጅዎ የሚሰጠው የኳርተም መድሐኒት</u>**

የካርተም መድሐኒት በውል ተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታል፡፡ ሆኖም ይህ ከጥናቱ ጋር የሚያገናኘው አንዳች ነገር የለም፡፡ የመድሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻለው እየባሰበት የሚሄድ ታካሚ ከተገኘ በወባ የህክምና መመሪያ መሰረት በተሻለ መድሐኒት እንዲታከም ይደረጋል፡፡

7. <u>በጥናቱ በመሳተፉ ልጅዎ ያለው መብትና ጥቅም</u>

በዚህ ጥናት ውስጥ መካተት የሚቻለው በፈቃደኝነት ብቻ ስለሆነ ልጅዎ በጥናቱ ያለመሳተፍ ሙለ መብት አለው፡፡ ልጅዎ በጥናቱ ውስጥ አልሳተፍም በማለቱ የሚያጣው እንክብካቤም ሆነ ህክምና አይኖርም፡፡ በጥናቱ ውስጥ መሳተፍ ከጀመረ በኋላም ቢሆን እንኳ ከጥናቱ መውጣት ቢፈልግ ሙለ መብት ያለው በመሆኑ የሚያስፈልገውን ህክምና በሙለ አንዴም ሳይጎልበት ከጤና ጣቢያው ያገኛል፡፡

በሌላ በኩል በዚህ ጥናት ውስጥ የሚታቀፍ ማንኛውም ታካሚ በተሰናዳው መርሃ ግብር መሰረት ወደ ጤና ተቋሙ ሲመላለስ ለእያንዳንዱ ቀን የመሄጃና የመምጫ የመጓጓዣ አበል ይሰጠዋል፡፡ በተጨማሪም ጥናቱ ከታካሚው የሚያገኘውን መረጃ ፍጹም ምስጢራዊነት የሚጠብቅ ሲሆን ከጥናቱ ጋር ተያይዞ በሚወጣ ማንኛውም ዓይነት ሪፖርት ውስጥ የታካሚው ስም አይጠቀስም፡ ፡

ከላይ የተዘረዘሩትን ጥናቱን የተመለከቱ መረጃዎች በሚገባ መረዳትዎን ያረጋግጡ፡፡ ያልገባዎት አረፍተ ነገር ወይም ግልጽ ያልሆነ ሃሰብ ካለም ማንኛውንም ማብራሪያ መጠየቅ ይችላሉ፡፡ ሁሉም መረጃዎች በሚገባ ግልጽ ከሆነልዎትና በጥናቱ ልጅዎ እንዲታቀፍ የሚፈቅዱ ከሆነ ከስር የተቀመጠውን የስምምነት ሰነድ ተረድተው ይፈርሙ፡፡

ለ/ <u>የስምምነት ሰነድ</u>

ጥናቱን የተመለከተውን መረጃ አንብቤ/በቋንቋዬ ተነቦልኝ ይዘቱን በውል ተገንዝቤአለሁ፡፡ አስፈሊጊ ሆኖ ካገኙት በፈለኩት በማናኛውም ጊዜ ልጄ ጥናቱን የማቋረጥ መብት እንዳለውም በሚገባ አውቄአለሁ፡፡ መረጃውን ካነበብኩ/ከተነበበልኝ በኋላ ያልገባኝን ነገር ጠይቄና ተረድቼ ልጄ በጥናቱ እንዲታቀፍ በፈቃደኝነት ተስማምቻለሁ፡፡

የጥናቱ ተሳታፊ ስም _____ፊርማ _____ቀን ----------
የምስክር ስም _____ፊርማ _____ቀን ----------
የጥናት አድራጊው ስም _____ፊርማ _____
ቀን ----------

Assent Form for Children (Aged 8-17 years)

Title of the project: therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated *plasmodium falciparum* in Asayita district, Northeast Ethiopia.

Name of principal investigator: _____

Address: _____

Your doctor has concluded that you have *P. falciparum*-induced malaria and that you require therapy with a drug called Artemether-lumefantrine. The FMOH presently recommends Artemether-lumefantrine as the first line medication for the treatment of uncomplicated *P. falciparum* malaria in Ethiopia. This medical facility will give you free Artemether-lumefantrine medication for your malaria illness regardless of whether you choose to take part in the study.

If you could volunteer for this research project, which will enable us to closely monitor and record the progression of your malaria infection following treatment with AL, we would greatly appreciate your assistance. The AHRI carries out TES on a regular basis to verify the continued effectiveness of approved malaria therapies. Asayita is one of the six sites chosen for this study, where we plan to evaluate the effectiveness of Artemether-lumefantrine in treating malaria. The results of this study should assist administrators of national malaria control programs in assessing whether there is evidence of drug resistance to AL and whether alternative drugs would be needed in place of AL.

We are inviting all malaria patients aged 6 months and over living in this area to take part in this study.

If you agree, you will be treated with six doses of Artemether-lumefantrine given twice daily for 3 days. (This is the same treatment that you would receive if you decide not to volunteer for this study.) The first dose will be given at the clinic supervised by study nurse and the 2nd dose will be given to you to be taken at home. The study will take place over 28 days. During that time, you will be asked to come to the health facility on scheduled days 1, 2, 3, 7, 14, 21 and 28. You will also be asked to come to the clinic at any other time if you become sicker, develop new symptoms, or if you fail to get better. Transportation fees will be provided to you during each scheduled study follow-up visit. During each follow-up visit, we would like to obtain a finger prick blood samples from you by a qualified technician that would be used only for malaria diagnosis, to detect the presence of markers for malaria drug resistance, and to see the outcome of treatment. There is no

serious risk in participating, but you may experience a small pain during finger pricking. The pain should disappear within 1 day.

The Artemether-lumefantrine medicine can have some unwanted side-effects or some effects that we are not currently aware of; however, we will follow you closely and ensure proper medical treatment. If you take Artemether-lumefantrine as directed, the course of your illness and possible side effects from Artemether-lumefantrine should not be any different whether you volunteer for this study or not.

The Artemether-lumefantrine medicine may have some unexpected effects; however, we will follow you closely and keep track of these effects, if they arise. Patients showing deterioration in their clinical status will be immediately admitted to the clinic free of charge for appropriate treatment according to the national policy till they recover. A physician will be responsible for every trial related medical decision of the patient throughout the study period.

Your participation in this study is completely voluntary and you can refuse to participate or are free to withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or all the services you receive at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive at the clinic will continue. If you decide to participate in this study, the information in your records is strictly confidential and your name will not be used in any report and any illnesses related to malaria or to the malaria treatment will be treated at no charge to you.

Do you understand what has been said to you? If you have any questions you have the right to get proper explanation.

Certificate of Assent

I have been invited to participate in a Artemether-lumefantrine effectiveness study. I have read the information in this assent form or has been read out to me in my language. I clearly understand the content. I am also aware of my right to optout of the study at any time during the course of the study without having to give reasons for doing so. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction and I voluntarily consent to participate in the study

Name of participant: _____

Signature of participant: _____

Date: _____

(dd/mm/yyyy)

Witness' signature: I confirm that the participant has given consent freely.

Print name of witness: _____

Signature of witness: _____

Date: _____

(dd/mm/yyyy)

Investigator's signature:

I confirm that the participant has given consent freely. Print name of investigator: _____

Signature of investigator: _____

Date: _____

(dd/mm/yyyy)

ለልጆች የተሰናዳ የስምምነት ቅጽ

ሀ/ ጥናቱን የተመከቱ መረጃዎች

1. የጥናቱ ርዕስ

የፐላዝሞዲየም ፋልሲፓረም ህክምና ጥቅም ላይ ሲውሌ 19 ዓመታት ያስቆጠረውን የኳርተም መድሐኒት ፍቱንነት ማጥናት፡፡ (Therapeutic effectiveness of Artemether-lumefantrine for the treatment of uncomplicated *plasmodium falciparum* in Asayita district, northeast Ethiopia).

ጥናቱን የሚያጠናው ተመራመሪ ስም፡- ----------

አድራሻ፡- _____

2. **<u>የጥናቱ ዓላ</u>**፡-

ኳርተም የወባ መድሒኒት የፐላዝሞዲየም ፋልሲፓረም ወባ ህክምና የሚሰጥ የመጀመሪያ ደረጃ መድሐኒት ነው፡፡ የፌደራል ጤና ጥበቃ ሚኒስቴር የወባ መድሐኒቶችን ፍቱንነት በየጊዜው በሚደረጉ ጥናቶች ይፈትሻል፡፡ ይህ ጥናትም የዚህ አይነቱ ፍተሻ አካል ሲሆን በአርማዎር ሀንሰን የምርምር ተቋም የሚካሄዴ የካርተም መዴሐኒት ፍቱነንት ፍተሻ ጥናት ነው፡፡

ይህ ጥናት በ6 የኢትዮጵያ አካባቢዎች ወባን በመፈወስ ረገድ ኳርተም ምን ያህል እየሰራ እንደሆነ በመገምገም መድሐኒቱ አሁንም ድረስ ፍቱን ሆኖ መዝለቁንና አለመዝለቁን ማረጋገጥ የሚደረግ ሲሆን ከጥናቱ የሚገኘው መረጃ ወባው መድሐኒቱን መላመድና አለመላመዱን መለየት ብሎም የመድሐኒቱ ምትክ ማስፈለጉንና አለማስፈለጉን ለመወሰን ጥቅም ላይ ይውላል፡፡ የጥናቱ ዓብይ ዓላማም ይሄው ነው፡፡

3. **<u>በጥናቱ የሚታቀፉ ታካሚዎች</u>**

ይህን ጥናት ለማጥናት እድሜያቸው 6 ወር እና ከዛ በላይ የሆናቸው የወባ ታማሚዎች በበጎ ፈቃደኝነት ተሳታፊ ይሆናለ፡፡ ከዚህ አኳያ እርሰዎ በኳርተም መድሐኒት በሚታከም ወባ ሳቢያ መታመምዎ በምርመራ ከተረጋገጠ በጥናቱ እንዲካተቱና ለጥናቱ እገዛ እንዲያዯርጉ የእርሰዎ ፈቃድ ያስፈልጋል፡፡

4. **<u>እርሰዎ ፈቃድዎን ገልጸው በጥናቱ ከታቀፉ በጥናቱ የሚሳተፉት እንዴት ነው?</u>**

በጥናቱ ላይ ለመሳተፍ ከተስማሙ፣ በቀን ሁለት ጊዜ ለ 3 ቀናት በሚሰጥ 6 የ Artemether-lumefantrine መጠን ይታከማሉ። (ለዚህ ጥናት በበጎ ፈቃደኝነት ላለመሳተፍ ከወሰኑ እርስዎ የሚወስዱት ተመሳሳይ ህክምና ነው።) የመጀመሪያው ልክ መጠን በጥናቱ ላይ በሚሳተፍ የህክምና ባለሙያ ክሊኒክ የሚሰጥ ሲሆን በቀን 2 ጊዜ ለ3 ቀናት የሚሰጥ ሲሆን በቀጣዩ ቀናት ጧት ጧት ወደ ጤና ጣቢያው እየመጡ መድሐኒቱን ይወስዱና ማታ የሚዋጠውን ደግሞ ተቀብል ወደ ቤቶ ይመለሳሉ፡ ፡ ጥናቱ በ28 ቀናት ውስጥ ይካሄዳል። በዛን ጊዜ 1, 2, 3, 7, 14, 21 እና 28 ወደ ጤና ተቋም እንዲመጡ ይጠየቃሉ. በተጨማሪም በማንኛውም ጊዜ ከታመሙ ወደ ክሊኒኩ እንዲመጡ ይጠየቃሉ፡፡ መድሐኒቱን ወስደው ሳይሻልዎት ቢቀርና አዲስ የወባ በሽታ ምልክት ቢያሳዩ ከመርሀግብሩ በተጨማሪ በማናቸውም ወቅት ወደ ጤና ጣቢያው እንዲመጡ ተደርጎ ክትትል እየተደረገልዎት ይታከማለ፡፡

5. **<u>በጥናቱ ወቅት ለእርስዎ የሚደረጉት የወባ ምርመራዎች</u>**

በጥናቱ ውስጥ ህክምናውን በሚከታተሉበት ወቅት ከጣት ላይ ደም እየተወሰደ የወባ ምርመራ ይደረግልዎታል፡፡ ከጣት ደም ሲወሰድ ጣት ስለሚበጣ ጥቂት ለአጭር ጊዜ ብቻ የሚቆይ የህመም ስሜት ይኖረዋል፡፡ በተወሰደው ደም የሚደረገው ምርመራ እየተሻለዎ መምጣቱን እንዲሁም ወባው ለመድሐኒቱ ያለውን ብግርነት ያሳያል፡፡

6. **<u>ለእርስዎ የሚሰጠው የኮአርተም መዲሐኒት</u>**

የኳርተም መድሐኒት በውል ተለይቶ ያልታወቀ የጎንዮሽ ጉዳት ሊኖረው እንደሚችል ይገመታሌ፡፡ ሆኖም ይህ ከጥናቱ ጋር የሚያገናኘው አንዳች ነገር የለም፡፡ የመዲሐኒቱን ተጽእኖ በማጥናት ሂደት ሳይሻልው እየባሰበት የሚሄዴ ታካሚ ከተገኘ በወባ የህክምና መመሪያ መሰረት በተሻለ መድሐኒት እንዲታከም ይደረጋል፡፡

7. **<u>በጥናቱ በመሳተፍዎት ያልዎት መብትና ጥቅም</u>**

በዚህ ጥናት ውስጥ መካተት የሚቻለው በፈቃደኝነት ብቻ ስለሆነ በጥናቱ ያለመሳተፍ ሙለ መብት አልዎት፡፡ በጥናቱ ውስጥ አልሳተፍም በማለትዎ የሚያጡት እንከብካቤም ሆነ ህክምና አይኖርም፡፡ በጥናቱ ውስጥ መሳተፍ ከጀመሩም በኋላም ቢሆን እንኳ ከጥናቱ መውጣት ቢፈሌጉ ሙሉ መብት ያልዎት በመሆኑ የሚያስፈልገውን ህክምና በሙለ አንዴም ሳይጎልብዎት ከጤና ጣቢያው ያገኛለ፡፡

በሌላ በኩል በዚህ ጥናት ውስጥ የሚታቀፍ ማንኛውም ታካሚ በተሰናዳው መርሃ ግብር መሰረት ወደ ጤና ተቋሙ ሲመላለስ ለእያንዳንዱ ቀን የመሄጃና የመምጫ የመጓጓዣ አበል ይሰጠዋል፡፡ በተጨማሪም ጥናቱ ከታካሚው የሚያገኘውን መረጃ ፍጹም ምስጢራዊነት የሚጠብቅ ሲሆን ከጥናቱ ጋር ተያይዞ በሚወጣ ማንኛውም ዓይነት ሪፖርት ውስጥ የታካሚው ስም አይጠቀስም፡

ከላይ የተዘረዘሩትን ጥናቱን የተመለከቱ መረጃዎች በሚገባ መረዳትዎን ያረጋግጡ፡፡ ያልገባዎት አረፍተ ነገር ወይም ግልጽ ያልሆነ ሃሰብ ካለም ማንኛውንም ማብራሪያ መጠየቅ ይችላሉ፡፡ ሁሉም መረጃዎች በሚገባ ግልጽ ከሆነልዎትና በጥናቱ እንዲታቀፉ የሚፈቅዱ ከሆነ ከስር የተቀመጠውን የስምምነት ሰነድ ተረድተው ይፈርሙ፡፡

ለ/ **<u>የስምምነት ሰነድ</u>**

ጥናቱን የተመለከተውን መረጃ አንብቤ/በቋንቋየ ተነቦልኝ ይዘቱን በውል ተገንዝቤአለሁ፡፡ አስፈሊጊ ሆኖ ካገኘሁት በፈለኩት በማናኛውም ጊዜ ጥናቱን የማቋረጥ መብት እንዲለኝ በሚገባ አውቄአለሁ፡፡ መረጃውን ካነበብኩ/ከተነበበልኝ በኋሊ ያልገባኝን ነገር ጠይቄና ተረድቼ በጥናቱ እንድታቀፍ በፈቃደኝነት ተስማምቻለሁ፡፡

የጥናቱ ተሳታፊ ስም _____ፊርማ _____ቀን ----------

የምስክር ስም _____ፊርማ _____ቀን ----------

የጥናት አድራጊው ስም _____ፊርማ _____ቀን ----------

Kasleh. Massoysen Ittingeyn.kukta

H|A cubbuss. Wagttta oytitte

1. **Akusaaqih. Cubbussoh.Ammunta.**
   - Plaazmudyem. Falcipharam (cangal Qaso/moyya Qaso) Aqasoh daylih.diwaata.tuxxiqi| Assay.19. Sanootah. Qumri. Lehtan Coartem (Quartam) xiqsitta diwa.biyak ursiyyihdudih.cubbusso.
   - Theya putic effecteve ness of Artemser Lumefantrine for the threatment of uncomplicated *P. falciparum* in Asayita district north east Ethiopia
   - Adiwak. Tu-xxiq Xalot Artemser Lumefantrine xiqitta diwaata Caddo Akak.fayyeyya AX Ce wayte- Gangal Qaso (moyyaqasoh). Aysaqiitak.Naharsi Caddoh. Ayromawqah. Ittopiyat raaqa hosptala.
   - Akusaq kusaaqisa.cubbuseh Migaq _____
   - Guubu _____
2. **Akusaaqah hadafa**
   - Coartem xiqsitta diwa (cangal Qasoh) tamcawwe naharsi caddoh dawa kinni. federaalak. Qalmalak. Qaso diwaatah.XaLot duddih cubbusiyyay Kusaqay udduruk Abah Iyya. Akusaq kaadu kusaaqitte k kusaaqittek.Tiyakteena kinni waka.
   - Armueer Hansan. Kusaq fanteena Abtah tan Coartem. diwak. Qaffoysiyyi duddih kusaq kinni.
   - Akusaq 6.Ethiopiah. deraafey. Qaso biyaa kih diwaduddi duddi yaklkh yan kusaq. Asmatuk geyna oyititte diwa barite Qaso kinnik Akkewa ytam baxisun Axcihih milaagufaxxa diwa kinnikAkkewayam. Madqimtamtam fan Tu-xxiqil-Asele.
   - Akusaqak.Lafelehadaf Thkinni
3. **Kusaq Edde yakke daylih mara**
   - -Akusaq wagta mari qumrik.6, Alsak.Elaa Idoola qaso biyak ellegeytime marakee meqem fayximar akinni., Tohuk Amakkaquk Atu. Coartem diwat day cimta Qasa kolgeytimtek. Akusaaqat. gaba tassa gallu kee qokol takku. Oggolte
4. **Atu feyixik oggoltek.kusaaqat elletangalenna?**
   - Akusaaqat tengelek. Ayroh. Adda l 2, Adda 3, Ayroh diwa.beetto.
   - Ameqem feyxit.tengelek.Atu.tonnah.tan diwa kaccenno caddok tonnay todaylih Aracay. Ayrol fan teena kah tabaatennah esserra, ossotinah aysewaytek. fanteenal koo kattaatak Akah day vintannah

5. **Kusaq uddur kohaban qasofokaqo**

- kusaqAddat. Yakkeh yan day li kattat. Feerak beynah nan. qabalak.kusaq Abna.
- Feerak qabal beyan uddur dago waqdi suga dananow Leh.ben qabalak Abna kusaq Sahall owak Akah yabattennah.Qaso diwak. Ataaleh.teysem tay bulle.

6. **Koh yaceen Qasô diwaata.**

- Coartem diwa.tu-xiq keetu-kalet duddah.
- Takkay Ikkah. Akusaq kee. diwa duddliboola·baxsale qexsite kee Aysewee. biyak Lafakkaalafek. Yankaatekkek . Qaso daylih diwatah medqal yayse daylat daylima.

7. **Akusaqat Abtagabat Agle·lito garkee Tuxig**

- Akusaaqat tangalem dudamfayk xi Lenum dubuk kinnimih taagah. Faxeweek Angalewaam duudah.
- Akusaaqat mangal Iyyaamah- daylakeehanaawi.nelleh.
- Akusaqih. Addat yangalayAngaleway fanak cabam duudah. Faxxitimmi Iyyah yan dayla Inkiih kaah Abna.
- gersi katuk.Akusaaqih Addat. yan gzleh yan mari Inkiih. Keenihmassosne kannaba
- gexoy madara. Marayti Noolih Assenta keenih Abak.
- Tohullih biyaakita. num yeeh yan oyti., Kusaq Abekee kayfanat raaqa.
- Akusaaqih Addat qaxe oyta ycaay kay migaq mahayna.
- Adagal Edde yabba Inne kusaq aytita cedekwoytam yasmiteenimi
- Kaah. Qaddo we wayte qangara tenek.kaahIffeysaanama
- Inkiih . yan oyti Qidi keenihi baahek lakalkusaaqat. Yangaloonuh. oggol Abeenik . Agubalfiirisen Ittingeysumaqta hayis.

L. Ittingey. Ayyufta.

Akusaq wagga hayta oyttite. Yaa fat yohah rayuk. Cedeeklakat faxximaksugtak.Akusaqahattaweqemduudaamih. gar Liyom Aaxaguk. Akusaaqih oytit gaba Assagalluh yefaxi kennimih samaqta heeh Sumaqta _____

Kusaqati yan numih migaq_____ Ayro _____

Sumaqta _____ Kusaq Abe miqiq _____

Ayro _____ Sumaqta _____

Wade migaq _____ Ayro _____

DECLARATION

I hereby declare that this MSc. thesis report is entirely my own work and has not been submitted elsewhere. I have acknowledged all the sources used in this proposal write-up.

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Examiners:

1. _____ Signature _____Date__/___/____
2. _____ Signature _____Date___/___/___

Place of submission: Department of Microbiology, Immunology and Parasitology, School of Biomedicine And Laboratory Sciences, College of Health Science, Addis Ababa University.

Date of Submission: _____/____/_____

Department of Microbiology, Immunology andParasitology (DMIP)

Department Research Ethics Review Committee (DRERC)

Meeting No: DRERC/001/2024 Date: 07 Feb 2024

Protocol Title: Therapeutic effectiveness of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Asayita district, Northeast Ethiopia

Principal Investigator	Alemayehu Sintayehu	
Institute/Department	CHS-AAU DMIP	
Type of review	[x] Initial Review	[ ] Amendment [ ] Other (specify)____
Elements Reviewed	[ ] Attached	[ ] Not attached
Decision of the meeting	[x] Approved	[ ] Approved with Recommendation
	[ ] Revision requested	[ ] Disapproved
Action Required	[ ] Send to IRB	[x] Authorize Implementation

Obligations of the PI:

i. Should comply with the standard international and national scientific and ethical guidelines
ii. All amendments and changes made in protocol and consent form needs DREC approval
iii. The PI should report Serious Adverse Events (SAE) within 10 days of the event
iv. End of the study, including thesis work and manuscript should be reported to the DREC

Follow up report expected in:
3 Months_____ 6 Months _X__ 9 Months _____ one year _____

Asrat Hailu (Prof)
Chair, DRERC
Signature
Date 07/02/2024

Department Stamp

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FEDERAL DEMOCRATIC REPUBLIC OF ETHIOPIA
MINISTRY OF EDUCATION

ቀን/DATE: 13 OCT 2023
ቁጥር/REF NO: 17/157/918/23

Armauer Hansen Research Institute (AHRI)
Addis Ababa

Subject: Letter of Approval

The Ministry Education (MoE) via its National Research Ethics Review Board has reviewed **"Ethiopia in vivo Efficacy Study 2023: Therapeutic efficacy of Artemether-Lumefantrine (AL) for the treatment of uncomplicated Plasmodium falciparum."** Project protocol in an expedited manner. We are writing to advise you that MoE has granted full approval to the above named project for a period of one year, from(**October 12, 2023 - October 11, 2024**).

All your most recently submitted documents have been approved and the study should comply with the international and national scientific and ethical standard guidelines. Any change to the approved protocol or consent material must be reviewed and approved through the amendment process prior to its implementation. In addition, any adverse or unanticipated events should be reported within 24-48 hours to MoE. Please ensure that you submit biannual progress report to MoE once in six months and annual renewal application 30 days prior to the expiry date.

We, therefore, request you as PI (Copied) and your esteemed organization to ensure the commencement and conduct of the study accordingly and wish for the successful completion of the project.

Sincerely

Solomon Tenor Belay (PhD)
Chief Executive Officer
Research and Community Engagement

Cc
- State Minister for Higher Education Development Sector
- CEO, Research and Community Engagement Affairs
- Research Ethics Desk
  Ministry of Education
- Dr. Fitsum Girma (PI)
  AHRI