Chapter 1

Introduction

1.1 Background

Plasmodium falciparum is a parasite that is known to induce human malaria. *P. falciparum* enters the human body via mosquitoes as its mode of transmission. The parasite endures the exo-erythrocytic cycle, which is centered in the liver, and the erythrocytic cycle, which circulates around red blood cells (RBCs) within the human body. In the erythrocytic cycle, it was known that the parasite had multiple stages that could be observed and analyzed with the aid of disciplines such as microscopy. These stages include the ring stages, which are characterized by small clusters of immature trophozoites within the RBCs, the mature trophozoite stage, which is characterized by a larger cluster of parasites, and the schizont stage, which is frequently observed as clusters of small parasite clusters (Singh & Daneshvar, 2013).

There were an estimated 247 million malaria cases and 619 thousand malaria-related deaths worldwide in 2021 with the majority of cases concentrated in rural tropical regions, demonstrating the alarming nature of the malaria threat. Nearly half of the world's population was at risk of contracting malaria (World Health Organization, 2022). In addition, Maude et al. (2019) reported that the Asia-Pacific region, where an estimated 2.31 billion people are at high risk of contracting malaria. With this alarming nature in mind, a plan by the OECD/WHO (2022), seeks to eradicate malaria by 2030 though the results of this program remains to be seen. This goal could be attained through the implementation of two primary strategies: vector control, which seeks to reduce the likelihood of mosquito bites, and the management of malaria cases through the use of rapid diagnostic tests and artemisinin-based combination therapies (ACTs) (Yu et al., 2023).

Diagnosis of *P.falciparum* remains one of the most important and effective stages in the treatment and control of *P.falciparum* as an early and accurate diagnosis of the presence of *P.falciparum* leads to a more efficient treatment process which significantly decreases the threat of the *P.falciparum* infection (Zekar & Sharman, 2020). Traditionally, in areas where malaria is endemic, Leishman, Giemsa, or Romanowsky stained peripheral blood materials and smear examination is the gold standard for diagnosing malaria. Microscopy has the benefits of being sensitive, educational, reasonably priced, providing a permanent record, and can be shared with other disease control programs. On the other hand, it has drawbacks including being labor- and time-intensive. Rapid techniques including the AO stain, quantitative buffy coat, and the detection of soluble HPII antigen in whole blood were also frequently used in the detection of *P.falciparum*. Following Clinical examination, microscopic examination is done to further help the diagnosis of P. falciparum (CDC, 2023). In addition, serology and antigen tests are generally the second line diagnosis method performed as confirmation tests when microscopic examination fails to identify the pathogen. Although this established detection method offers straightforward procedures, it is frequently challenging to determine whether the parasite is resistant to any drug treatment. The determination of drug-resistant strains occurs after the patient has been treated with the drug and their health has not improved significantly (Wicht et al., 2020). Detecting these drug-resistant parasites early would enhance the likelihood of effective treatment.

According to genomic and proteomic analyses, drug-resistant P. falciparum is associated with critical regulatory genes. The PfCRT locus is associated with chloroquine resistance (Ecker et al., 2012). Single nucleotide polymorphisms (SNPs) in Plasmodium falciparum have been increasingly recognized as key contributors to the development of drug resistance, particularly against antimalarial therapies. Resistance to artemisinin-based combination therapy (ACT) is caused by polymorphisms in the pfMDRT and/or kelch13 loci (Bwire et al., 2020; She et al., 2020). The most well-known gene associated with artemisinin resistance is pfk13. Specific mutations within this gene, such as C580Y,

have been correlated with delayed parasite clearance rates following artemisinin-based combination therapy (ACT). Studies have demonstrated that these mutations lead to alterations in the PfKelch13 protein that disrupt normal cellular processes, allowing parasites to survive despite exposure to artemisinin derivatives By detecting these polymorphisms, drug-resistant *P. falciparum* strains can potentially be identified.

This project's primary objective is to create a novel diagnostic kit based on isothermal amplification and later CRISPR technology that targets vital *P. falciparum* genes that are associated with drug resistance.

1.2 Objective

The goal of this research is to optimize a method for amplifying P. falciparum genes related to drug resistance utilizing an isothermal approach, of which specificity and sensitivity are assessed and compared to PCR.

1.3 Hypothesis

LAMP amplifies DNA more quickly and efficiently than PCR, making it possible to amplify tiny amounts of DNA. This is because it amplifies constantly at a constant temperature, perhaps increasing its sensitivity to low-abundance target detection. High specificity can be obtained by utilizing multiple primers (often six) that each recognize a different part of the target sequence.