I. INTRODUCTION

1.1. Background

Malaria is a severe disease that is caused by the parasite from the genus *Plasmodium*. The disease is transmitted by the bite of the infected female *Anopheles* mosquitoes, allowing parasites into the bloodstream (Talapko et al., 2019). Malaria has been a major health issue around the world for many years, with tropical and subtropical countries being mostly endemic to the disease (Mussa et al., 2019). In Indonesia, there were around 800,000 cases of malaria recorded in 2021 (Sugiarto et al., 2022; Guntur et al., 2021). Thus, a major pillar in the control and elimination of malaria are diagnostic methods.

There are various different approaches to detect malaria. The most common diagnostic methods to detect malaria are microscopy and rapid diagnostic tests (RDTs) (Maltha et al., 2013; Kavanaugh et al., 2021). However, microscopy is not recommended in areas where its expertise is lacking, such as rural areas (Kozycki et al., 2017). Therefore, an alternative method is RDTs. There are different types of RDTs, such as PfHRP2-based (*Plasmodium falciparum* histidine-rich protein 2), and PLDH-based *(Plasmodium* lactate dehydrogenase) RDTs. Among the two, the most widely used RDT is the PfHRP2-based, as it has a higher sensitivity and thermal stability compared to PLDH-based RDTs (Mouatcho & Goldring, 2013).

The antigen that is targeted in PfHRP2-based RDTs is histidine-rich protein 2 (HRP2), abundantly produced in asexual stages of *Plasmodium falciparum*. Thus, it is frequently used as a biomarker for RDTs to detect *P. falciparum* (Mussa et al., 2019; Abdallah et al., 2015). The protein is encoded by the *pfhrp2* gene, which is located in chromosome 8. A paralogous gene is also present, located in chromosome 13, known as *pfhrp3*. The gene has a similar amino acid sequence and epitope to the *pfhrp2* gene. Hence, PfHRP2-based RDT can cross-react with the pfhrp3 gene (Viana et al., 2017).

Although PfHRP2-based RDTs have been widely used, the quality and accuracy of this tool are being threatened by a mutation in *pfhrp2/3* genes.

Pfhrp2/3 gene deletions are mutations that occur in the chromosomes of the genes. This deletion is caused by the breakage and rejoining in the chromosomes, which leads to a segment of the gene or the entire gene to be deleted (Cheng et al., 2014). This mutation poses a major threat as it can lead to false negative RDT results, which can decrease the accuracy and sensitivity of PfHRP2-based RDTs, and delay the control and elimination of malaria (Amoah et al., 2020). Cases of this gene deletion have been recorded in many countries in recent years, including in Papua, Indonesia. However, there has yet to be any publications regarding the issue. In efforts to detect this gene deletion, studies have developed a strategy. One of the crucial steps prior to detecting *pfhrp2/3* gene deletions is the detection of single copy genes (Thang et al., 2022; Thomson et al., 2019).

Single copy genes are genes that exist as a single copy in a whole genome of an organism. These genes are not duplicated within a genome. Hence, they are typically used as biomarkers to determine the presence of the species (Wang et al., 2022). Examples of single copy genes that are specific to *P. falciparum* include GLURP, MSP1, MSP2, and EBA 175 genes. These genes play an important role in erythrocyte invasion, parasite growth, and immune system evasion (Pattaradilokrat et al., 2018; Low et al., 2007; Dijkman et al., 2021; Tolia et al., 2005). The detection of these genes can determine the presence and quality of the parasite DNA (Cheng et al., 2014). Therefore, the study proposes to detect the presence of *P. falciparum* single copy genes, specifically GLURP and EBA 175 genes, to determine the DNA quality of the parasite in samples collected from Papua, Indonesia.

1.2. Objective

The objective of the study is to detect *P. falciparum* single copy genes (GLURP and EBA 175 genes) in malaria samples collected from Papua, Indonesia.