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DNA QUALITY TESTING OF ISOLATED STORED GIEMSA BLOOD SMEAR SAMPLES USING GENEIUS™ MICRO gDNA EXTRACTION KIT AS AN ALTERNATIVE SAMPLE FOR MALARIA MOLECULAR EXAMINATION

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ABSTRACT
The gold standard for malaria examination is microscopic examination. However, microscopic examination has many shortcomings, namely the difficulty of distinguishing between Plasmodium sp. species when mixed infections occur. Other malaria examinations for screening can be done with RDT. However, it also has shortcomings, namely the minimum number of parasites that can be detected is 100 parasites/ul. Molecular examination is the most sensitive and accurate examination in the diagnosis of malaria. The initial stage of molecular examination is the DNA isolation stage. This DNA isolation is very important in determining the success of molecular examination. Isolation of Plasmodium sp. DNA using blood smear samples is still rarely done and there is no standard procedure for isolating DNA from blood smears. The purpose of this study was to determine whether stored Giemsa blood smears can be used as samples in DNA isolation in malaria cases. The method used in isolation is using the Geneius™ Micro gDNA Kit. The results of the study were that stored Giemsa blood smear samples of Plasmodium vivax for 3 months could be isolated using the Geneius™ Micro gDNA Kit and could be used as DNA templates for amplification with target gene primers.

Keywords: DNA isolation; DNA qualitative test; stored giemsa blood smear; malaria detection

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INTRODUCTION

Malaria is one of the infectious diseases that is still a health problem in the world. Malaria can be transmitted through the bite of the Anopheles sp. mosquito that carries the Plasmodium sp. parasite. Currently, there are five species of Plasmodium parasites that infect humans, including *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ova*le, and *Plasmodium knowlesi* (Ashley et al., 2018; Sambe et al., 2023). Malaria is endemic in more than 90 countries with an estimated 247 million cases and 619,000 deaths globally in 2021. Plasmodium falciparum infection is responsible for more than 90% of malaria deaths worldwide and is a threat to public health (Calderaro et al., 2024). Malaria diagnosis can be made using microscopic examination of blood smears (CDC, 2013; WHO, 2014). However, microscopic examination has many shortcomings that can reduce sensitivity, including poor Giemsa staining, difficulty in distinguishing between Plasmodium sp. species when a mixed infection occurs (Adel et al., 2014), the need for a laboratory technician who is skilled in microscopic reading (Puasa & Kader, 2018) and can only detect parasites within a threshold of 50-100 parasites/ul (Hutami, 2018). This method becomes inefficient in patients who experience Plasmodium infection without symptoms (Panda et al., 2019). Another diagnosis that can be used for screening is the Rapid Diagnostic Test (RDT). The malaria RDT examination is a rapid examination based on the detection of parasite proteins with the principle of immunochromatography (WHO, 2017). The advantages of RDT are affordability

and relatively short examination time so that treatment can be given immediately (Bastiaens et al., 2014). However, it has a drawback, namely the minimum number of parasites that can be detected by RDT is 100 parasites/ul (Aschar et al., 2022).

Molecular examination approaches are currently increasingly developing and widely used. Molecular examination is the most sensitive and accurate examination in the diagnosis of malaria. This examination can be used to diagnose parasitemia submicroscopically, detect resistance to anti-malarial treatment, and distinguish relapse from recurrent infection (Mann et al., 2015; Wang et al., 2014). The initial stage of molecular examination is the DNA isolation stage. This DNA isolation is very important and is one of the points of success of molecular examination. DNA isolation is carried out to obtain pure DNA with high concentration so that it can be used for further molecular analysis such as PCR, PCR RFLP, PCR RAPD, PCR ARMS, Nested PCR and so on (Sirait & Sulistiawati, 2023). The success of the DNA isolation process depends on the sample preparation carried out, where good sample preparation can produce good quality DNA isolation results (Wasdili et al., 2022). There are three main steps in DNA isolation, namely cell wall destruction (lysis), separation of DNA from other solid materials such as cellulose and protein, and DNA purification (Syafaruddin & Santoso, 2015).

Currently, many DNA isolation methods have been developed that can be done from whole blood samples, dry spots (filter paper) and blood smears. Based on previous research, Plasmodium sp. DNA isolation was carried out using EDTA blood samples (Seesui et al., 2018). The advantage of EDTA blood samples is that they have good quality but must be stored in a freezer at -20^{o}C. This method is difficult to do if sampling is in remote areas that do not have access to electricity and inadequate cooling equipment (Perwitasari et al., 2020). Taking blood samples using filter paper can also be done and is an alternative for taking blood samples in areas where facilities are lacking (Syaifudin et al., 2015). However, filter paper is not cheap and requires a lot of money to purchase and procure it. Meanwhile, isolation of Plasmodium sp. DNA using blood smear samples is still rarely done and there is no standard procedure for isolating DNA from blood smears. So it is necessary to do further research. The purpose of this study is to determine which method is most effective for isolating DNA from blood smear samples as an alternative sample for molecular malaria examination.

METHOD

The materials used in this study were Giemsa blood smears from patients infected with Plasmodium vivax, Geneius™ Micro gDNA Kit, absolute ethanol, loading dye, gel red, aquabidest, 1.5% agarose, TBE 1x. The equipment used in this study were centrifuge, micropipette, tips, electrophoresis, vortex, spindown, dry bath, gel documentation, microcentrifuge tube, and Eppendorf tube. This study was a non-experimental study, the samples used were 24 samples of Giemsa blood smears stored from patients infected with Plasmodium vivax. The samples used were samples stored for 12 months, 10 months, 9 months, 8 months, and 3 months. The sample preparation procedure was carried out by dripping the blood smear with ±100 µl PBS solution, after which it was scraped slowly using a sterile scalpel. The scraping results obtained were transferred to a 1.5 mL Eppendorf tube. Repeat the blood smear penetration with PBS until the blood smear is clean. The final result obtained was ±600 µl of blood smear scraping sample.

DNA isolation with the Geneius™ Micro gDNA Kit. The isolation method used follows the DNA isolation work protocol listed in the kit by pipetting 200 µl of the scraping sample into an Eppendorf tube then adding 20 µl of Proteinase K, then vortexing and incubating at 60oC

for 30 minutes (every 10 minutes in the vortex). After incubation, 200 µl of S2 Buffer was added and vortexed. After that, it was incubated again at 60oC for 20 minutes (every 10 minutes in the vortex). After incubation, the supernatant was transferred to a new Eppendorf tube then 200 µl of absolute ethanol was added and vortexed for 10 seconds. Next, place the GD Column in a 2 mL Collection Tube and centrifuge at 14,000xg for 1 minute. Discard the liquid and collection tube. Then place the GD Column in a new Collection Tube and add 400 µl W1 Buffer and centrifuge at 14,000xg for 30 seconds. Discard the liquid captured in the collection tube. Next, place the GD Column back in the Collection Tube and add 600 µl Wash Buffer and centrifuge at 14,000 xg for 30 seconds. Discard the liquid captured in the Collection Tube. Next, centrifuge the empty GD Column again at 14,000 xg for 3 minutes. In the final stage, transfer the GD Column to an Eppendorf tube then add 200 µl Elution Buffer Preheated and incubate for 3 minutes at room temperature. Next, centrifuge at 14,000 xg for 1 minute, discard the GD Column. The isolate obtained is ready to be used for further analysis. Electrophoresis is carried out for qualitative testing of DNA obtained using 1.5% agarose and 1x TBE buffer. Furthermore, electrophoresis is carried out for 30 minutes at a current of 90 Volts. The electrophoresis results are visualized with gel documentation. Analysis of research data in the form of electrophoresis results showing the presence or absence of DNA bands. The data obtained are analyzed descriptive.

RESULTS

Microscopic identification of the blood smear showed that the blood smear sample was in good condition and Plasmodium vivax infection was detected (Figure 1).

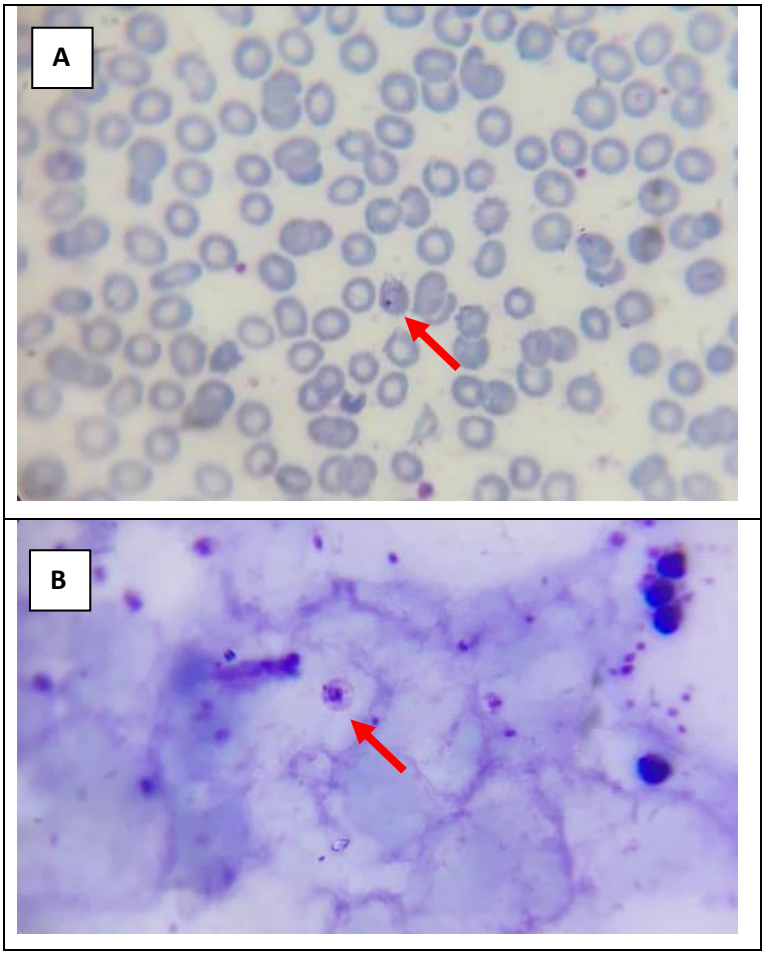

Figure 1. Microscopic view of thin blood smear (A) and microscopic view of thick blood smear (B).

Isolation was carried out by the initial stage of scraping the blood smear sample dripped with PBS solution using a sterile scalpel. Isolation was carried out using the Geneius™ Micro gDNA Kit. The isolation results were then electrophoresed to see the quality of the isolated DNA. The results of the qualitative test are shown in Figure 2, the DNA bands obtained were different for each sample, some were clearly visible, some were only very thin bands.

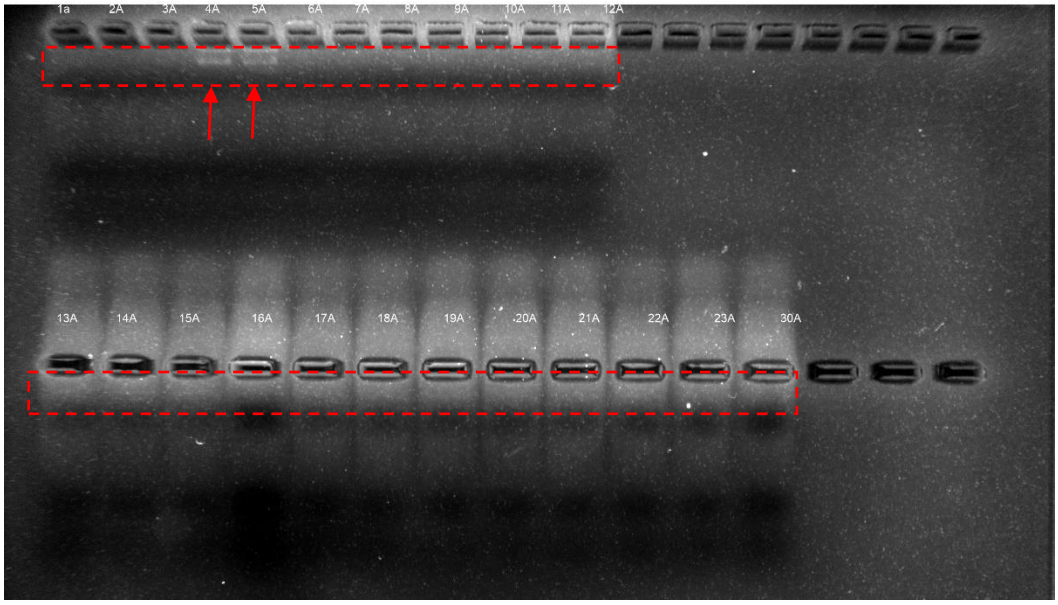

Figure 2. Visualization of electrophoresis results of qualitative DNA test of blood smear samples Sample 1A-24A (1.5% Agarose, 30 minutes, and 90V voltage). The bands are clearly visible in wells 4A and 5A, while the other wells are very thin and tend to be invisible.

DISCUSSION

The results of this study show a significant negative relationship between exposure to cigarette advertisements and adolescents' intention to quit smoking. Based on Pearson correlation analysis, it was found that the correlation value between exposure to cigarette advertisements and the inention to quit smoking was $r = -0.157$ with $p = 0.027$, indicating a significant relationship at a significance level of $p < 0.05$. This means that the higher the exposure of adolescents to cigarette advertisements, the lower their intention to quit smoking. These results are consistent with previous studies, which found that exposure to cigarette advertisements often reinforces positive perceptions of smoking behavior (Daulay et al., 2018; Hasanah et al., 2021). The finding that exposure to cigarette advertisements has a negative relationship with adolescents' intention to quit smoking implies that the more frequently adolescents are exposed to these advertisements, the less likely they are to intend to quit smoking (Andriati, 2021). A primary implication of this result is that cigarette advertisements do not only influence the intention to start smoking but also reinforce attachment to smoking habits, hindering efforts to quit (Daulay et al., 2018). These advertisements often associate smoking with positive images, such as freedom, a modern lifestyle, or masculinity, leading adolescents to perceive smoking as an integral part of their identity (Wijaya et al., 2017; Handaningtias, 2022). Consequently, adolescents frequently exposed to these advertisements find it more challenging to break free from the smoking habit, even if they are aware of the health risks involved.

This negative relationship aligns with previous research, such as that conducted by Shofa and Utami (2017), which indicated that cigarette advertisements can enhance emotional attachment to smoking. Advertisements often depict smoking as a socially accepted, even

advantageous activity, particularly in regions like Sumenep, where smoking traditions are strong. This finding also supports the research by Dewi et al. (2017), which revealed that cigarette advertisements significantly increase the intention to continue smoking among adolescents. In this context, exposure to cigarette advertisements not only hampers the intention to quit smoking but also reinforces social norms that support smoking behaviors.From a psychological perspective, this result can be explained through the Theory of Planned Behavior (Ajzen, 1991), which posits that positive attitudes toward a behavior, social norms (Rachmat et al., 2013), and perceived behavioral control influence the intention to engage in or refrain from a specific action. Cigarette advertisements play a crucial role in reinforcing positive attitudes toward smoking and social norms that support the behavior while simultaneously reducing the perceived self-control necessary to quit smoking (Zahrani & Arcana, 2021). In other words, cigarette advertisements create an environment where smoking is viewed as a behavior that is difficult to cease, further diminishing the intention to quit.

This study strengthens the findings of several previous studies, which state that cigarette advertisements, especially those presented visually through television, print media, and social media, can influence adolescents' attitudes and behaviors toward smoking. According to Tantri, cigarette advertisements have the ability to create a glamorous or masculine perception of smoking, which then reduces an individual's intention to quit smoking (Hasanah et al., 2021; Tantri et al., 2018). In the context of Sumenep, Madura, where the smoking culture is very strong, exposure to cigarette advertisements can further reinforce smoking habits as a socially accepted norm, thus hindering adolescents' efforts to quit smoking. Moreover, the results of this study show that the group of adolescents most exposed to cigarette advertisements are those with a high school education (76.5%). This indicates that older adolescents are more likely to be exposed to cigarette advertisements through the various media they frequently access, such as the internet and social media (Ramadona et al., 2024). This condition poses its own challenge in controlling smoking, especially since high school-aged adolescents are in a transitional phase toward adulthood and are more vulnerable to environmental influences, including media (Maharani & Harsanti, 2021).

Another finding from this study also supports the existing literature regarding the role of gender in smoking behavior. The majority of respondents in this study were male (70.0%), which is consistent with previous research showing that the prevalence of smoking among males is much higher than among females (Ridhoila et al., 2017). The strong masculine values in Madura’s culture may be one of the driving factors for smoking behavior among males, where smoking is often seen as a symbol of maturity and bravery. A gender-based analysis indicates that males tend to have higher rates of exposure to and prevalence of smoking compared to females, which can be explained through several social, cultural, and psychological factors. In many societies, including Madura, social norms often associate smoking with masculinity, social status, and bravery. Males, particularly adolescents, may feel pressured to demonstrate their masculine identity through behaviors such as smoking, which is frequently viewed as a symbol of maturity and social appeal (Hasanah et al., 2021; Ridhoila et al., 2017).

Research by Jufri et al. (2023) found that peer pressure also plays a significant role in influencing smoking behavior among adolescent males. When smoking peers are perceived as "cool" or accepted, adolescent boys may feel compelled to participate, increasing their exposure to cigarettes and cigarette advertisements. Additionally, according to a study by Maharani & Harsanti (2021), males are more likely to be exposed to cigarette advertisements

through social media and digital platforms, where ads often portray masculine images and modern lifestyles that appeal to them. From a psychological perspective, males are also more likely to hold positive attitudes toward smoking, reinforced by advertisements that depict smoking as a desirable behavior. Research by Tantri et al. (2018) indicates that positive perceptions of cigarette advertisements are directly related to the intention to smoke, particularly among adolescent boys. This suggests that exposure to cigarette advertisements not only promotes smoking behavior but also reinforces the perception that smoking is an integral part of their identity as males.

The results of this study have several important implications for the development of cigarette advertising control policies, especially in areas with a high smoking prevalence, such as Sumenep, Madura. One policy that could be considered is restricting cigarette advertisements in media easily accessible to adolescents, including social media and television, which are the main sources of advertisement exposure for this age group (Ama et al., 2021; Firmansyah et al., 2023; Hasanah et al., 2021). Additionally, further efforts are needed to provide education about the dangers of smoking specifically targeted at adolescents, as well as intervention programs that can increase adolescents' awareness and motivation to quit smoking. The findings of this study indicate that exposure to cigarette advertisements has a significant negative relationship with adolescents' intention to quit smoking, which presents several important implications for public policy and public health interventions. First, these findings highlight the urgent need to regulate and restrict cigarette advertising, particularly in media accessible to adolescents (Saleh et al., 2016). By reducing adolescents' exposure to cigarette advertisements, it is hoped that the positive influence these advertisements have on smoking behavior can be diminished. This aligns with recommendations from the World Health Organization (WHO), which calls for tobacco advertising control as a vital step in reducing smoking prevalence among adolescents (Morgenstern et al., 2013).Second, the results underscore the necessity for more effective education and awareness programs specifically targeting adolescents (Muflih et al., 2023). These programs should be designed to educate teenagers about the negative impacts of smoking and how cigarette advertisements can mislead their perceptions. Community-based approaches that involve families and community leaders can reinforce these messages and help create an environment supportive of smoking cessation efforts (Ramza, 2022). Peer-led interventions can also be effective, considering the strong influence of peer groups among adolescents (Wijaya & Rahman, 2020).

Third, policies should take into account the demographic characteristics of adolescents, such as gender and education level, when formulating intervention strategies. For instance, given that the majority of respondents were male (Zahrani & Arcana, 2021), programs should consider the social norms that may reinforce smoking behavior among adolescent boys and how cigarette advertisements perpetuate these norms. By gaining a better understanding of the factors influencing smoking behavior among adolescents, policies can be designed to be more effective in reducing smoking prevalence and increasing the intention to quit (Hasanah & Zahratul, 2022).This study has several limitations that need to be considered. First, the cross-sectional research design only allows for identifying relationships between variables at one point in time, so it cannot establish causal relationships. Second, the measurement of exposure to cigarette advertisements and the intention to quit smoking was based on self-reports, which may be prone to social and recall bias. Third, this study was limited to the Sumenep area, Madura, so the results may not be generalizable to other areas with different social and cultural contexts. For future research, it is recommended to use a longitudinal design to evaluate changes in the intention to quit smoking along with changes in exposure to cigarette advertisements over time. Additionally, further research needs to be conducted in other areas

in Indonesia to test whether these findings apply in a broader context. The use of more objective measurement methods, such as tracking media exposure, can also enrich the results of this study and minimize bias. DNA isolation is the most important step in molecular biology examination. DNA isolation and purification are basically a series of processes of separating DNA from other cell components. Extraction to obtain high-quality DNA is a basic rule that must be met in molecular analysis and is one of the success factors in DNA amplification that will be used in genetic character analysis (Mustafa et al., 2016; Restu et al., 2012). DNA isolation can be done by damaging or breaking the cell wall so that DNA comes out of the cell. The main stages in DNA isolation are cell wall destruction, separation of DNA from other components (protein, carbohydrates, fat) and DNA purification. Cell wall breakdown aims to destroy the membrane and cell wall so that the inside of the cell is easy to get out. DNA separation aims to separate DNA from other contaminants such as protein, RNA, lipids, and polysaccharides. Cell purification aims to remove residues used in the process of breaking the cell wall and separating DNA so that a truly pure DNA isolate is obtained without contaminants (Hutami et al., 2018). The quality of the DNA isolate obtained from the isolation process can be determined using agarose gel electrophoresis.

The results of the qualitative DNA test were obtained from 24 isolated samples, the results showing that the DNA bands appeared clearly and well only in wells 4A and 5A. The two samples were Giemsa blood smear samples from patients infected with Plasmodium vivax obtained from August 2024 so that the sample storage period was 3 months. While the other samples, the DNA bands appeared very thin and tended to be invisible. The other samples were Plasmodium vivax Giemsa blood smear samples obtained from patients in November 2023, January 2024, February 2024, and March 2024. The results obtained showed that the storage period greatly affected the quality of the DNA isolation results. The quality of DNA isolated from Giemsa blood smear samples that had been stored for more than 6 months showed that the quality of the DNA obtained was no longer good, while the quality of DNA from Giemsa blood smear samples with 3 months of storage still showed good DNA quality and the DNA bands were still clearly visible. In general, DNA isolation from stored Giemsa blood smear samples produces less and thinner DNA isolation results when compared to DNA isolation from whole blood samples. This can occur because the volume of blood in the Giemsa blood smear sample is very small. In addition, the effect of long storage time can also cause damage to blood cells including DNA being degraded naturally or by microorganisms (Putri & Junitha, 2015).

In the research conducted, the success factor of the DNA isolation process can be influenced by three main factors, namely the incubation temperature used, the length of incubation time, and the interaction between temperature and the length of incubation time (Inderiati et al., 2022; Perwitasari et al., 2020). Of the three factors, in this study, a modification was made to the incubation time, where the incubation time in the lysis process stated in the kit procedure was at a temperature of 60oC for 30 minutes. However, when the quality test results were visualized, the DNA bands obtained were very thin. So a modification was made by extending the incubation time to a temperature of 60oC for 60 minutes. The results obtained from this modification were that the visible DNA bands were increasingly visible. DNA must be of good quality so that the PCR process can run optimally. Therefore, the DNA isolation method has an important role in obtaining pure DNA with as few contaminants as possible (Donastin et al., 2022). Geneius™ Micro gDNA Kit is a reagent that can be used to isolate DNA from whole blood samples, dried blood spots (on FTA paper), urine, and swabs. Although the Geneius™ Micro gDNA Kit procedure does not state that it can be used to isolate stored Giemsa blood smear samples, this reagent can be used and is successful for isolating DNA

from stored Giemsa blood smear samples. The procedure used to isolate DNA from stored Giemsa blood smear samples can be done by following the procedure for isolating dried blood spots (on FTA paper).

CONCLUSION

Giemsa blood smear samples of Plasmodium vivax stored for 3 months can be isolated using the Geneius™ Micro gDNA Kit and can be used as DNA templates for amplification with target gene primers.

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